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Antivenom potential of *Matricaria pubescens* (Magnoliopsida: Asteraceae) against snakebite of the saharan horned viper *Cerastes cerastes* (Squamata: Viperidae) from North-East of Algeria

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Abstract
Matricaria pubescens (Magnoliopsida: Asteraceae) is an important medicinal plant used in Algerian folk medicine as an antiinflammatory, analgesic and antiseptic.
The present study was conducted on 16 males of Albino rabbits which were divided into four groups (a control group, a group envenomated by *Cerastes cerastes* venom, an envenomated group treated with venom and extract from medicinal plant *Matricaria pubescens*, and the last envenomated group treated with the anti-venom serum). Metabolic and histopathological changes were examined in different treated groups.
The histological sections from tissues of different organs (liver, kidneys, lungs, muscles, heart, and brain) allowed us to observe a normal structure in all the organs of untreated rabbits unlike the treated ones. Our results showed a very highly significant increase in the concentrations of glycemia, urea, and creatinine in treated rabbits compared to the control. statistical analysis showed that there was a very highly significant ($p = 0.0000$) between all the groups in the increase of glucose, urea concentration, TGO and TGP and a highly significant in the creatinine ($p = 0.0002$) and the PTT ($p = 0.0015$). On the other hand, a very highly significant decrease in the total cholesterol, triglyceride, fibrinogen, and Prothrombin time of treated rabbits compared to those of the control. Statistical data confirmed that there was a very highly significant ($p = 0.0000$) between all the groups. According to the PCA analysis, the biological parameters (cholesterol, triglyceride, fibrinogen, and prothrombin time) were very close, a certain similarity was reflected between them which means that these parameters were influenced by each other. Same thing for the glycemia, urea, creatinine, GOT, GPT, and PTT.

Keywords: *Matricaria pubescens; Cerastes cerastes;* antivenom; metabolism; histopathology

Introduction
More than 100000 venomous animal species identified in the world. Each of these species is capable of producing venoms that often contain upwards to 100 different molecules. Animal venoms are used effectively for defense and predation which are not composed of single toxins but cocktails of complex chemical mixtures of pharmacologically active components including proteins, peptides, and enzymes with specific biological activities, as well as some nonprotein compounds such as carbohydrates, lipids, metal ions and other, as yet, unidentified substances (Jimenez-Porras, 1968) [27]. Among these animals, we find snakes, where their snakebite envenoming caused a serious public health issue worldwide, including a range of acute medical emergencies, with deadly consequences where 81000 to 138000 people die every year from a snake envenomation, and 400000 victims/year live with permanent disabilities (WHO, 2021) [56]. On the other hand, there is a growing interest in studying snake venoms for therapeutic applications. In addition, current venomous snakes all belong to the Superfamily of Colubroids, which brings together about 2500 species of snakes (Chippaux, 2006) [15]. Meanwhile, Snake venom contains a mixture of cytotoxic, hypotensive, neurotoxic, and anticoagulant substances and their variability is considered at several levels interfamily, indigenous, interspecies, interspecies and intraspecies (Chippaux *et al*., 1991; Del Brutto & Del Brutto, 2012) [17, 18]. It also varied according to geographic origin, age, food preferences and breeding conditions (Rollard *et al*., 2015) [47].
The genus *Cerastes* (Laurenti, 1768) was the best identified and most abundant venomous snakes of the deserts of North Africa and the Middle East (Schneemann *et al*., 2004) [51]. This genus includes four species i.e., *Cerastes boehmei* (Wagner & Wilms 2010) [55], *C. vipera* (Linnaeus, 1758), *C. gasperettii* (Leviton & Anderson, 1967), and *C. cerastes* (Linnaeus, 1758), (Schleich *et al*., 1996; Baha El Din, 2006; Chippaux, 2006) [50, 5, 15]. The last species was responsible for many human snake bites (Schneemann *et al*., 2004) [51]. The largest species of *Cerastes* was the desert horned viper *Cerastes cerastes* (Linnaeus 1758). Its venom contains enzymes such as proteases (metalloproteinases, serine proteinases), L-amino acid oxidase, hyaluronidases, and toxins (cardiotoxins, myotoxins, neurotoxins) (Labib *et al*., 1979; Laraba-Djebari *et al*., 1992) [28]. On the other hand,

african traditional medicine contributes to meet the health needs of over 80% of the population (Rukangira, 1997) [48]. The Asteraceae family was rich in various natural components i.e., terpenoids, flavonoids, and alkaloids (Boutaoui, 2012) [14]. Among this largest family, *Matricaria pubescens*, also called Sahara chamomile or "Guertoufa", was used as a treatment against snake envenomation, this plant is very popular in algerian traditional medicine (Ould El-Hadj *et al*., 2003; Bouallala & Chehma, 2014) [13]. Where the aqueous extracts of their aerial part showed the presence of alkaloids, coumarins, saponins, flavonoids and terpenes (Benkiki 2006; Gherboudj *et al*., 2012; Makhloufi *et al*., 2012; Benferdjallah *et al*., 2019) [9, 22, 35, 8].

We extracted the venom of three individuals of this desert horned vipThe aim of our study was to know the effects of *Cerastes cerastes*' venom on the parameters and the biological disturbances observed during envenomation's viperine, and also to evaluate the anti-envenomation activity of *Matricaria pubescens*.

Material and Methods

1. Plant Material

Matricaria pubescens (Fig. 1A). is an aromatic spontaneous species of the Asteraceae family. It is an annual plant of 50 cm to 1.5 m tall, with an erect, branching stem. It is used to treat eye diseases, itching, inflammation of wounds, diseases of the female genital tract and bronchopulmonary diseases (Maiza *et al*., 1995; 2011, Hammiche *et al*., 2006) [34, 33, 25] digestive disorders, internal tumors, rheumatism and neuralgia (Djellouli *et al*., 2013) [19], Antihyperglycemic (Amssayef *et al*., 2020), scorpion envenomation (Adaika *et al*., 2021) [2]. The aerial part of this plant was harvested at flowering stage, from March to May 2018, from the North-Eastern region of El-Oued' province, Taleb El Arbi (Fig. 1B). It was dried in the shade and undercover at room temperature for few days. The extraction of flavonoids was carried out according to the method described by Bekkara *et al*., 1998 [7]. For liquid-liquid partitioning, ethyl acetate was the used solvent. *Matricaria pubescens* was subjected by maceration in a hot hydro-alcoholic mixture; after evaporation of alcohol, a liquid-liquid extraction was made for the aqueous phase with ethyl acetate, after evaporation to dryness. The obtained extracts were stored in the refrigerator for further analysis and applications.

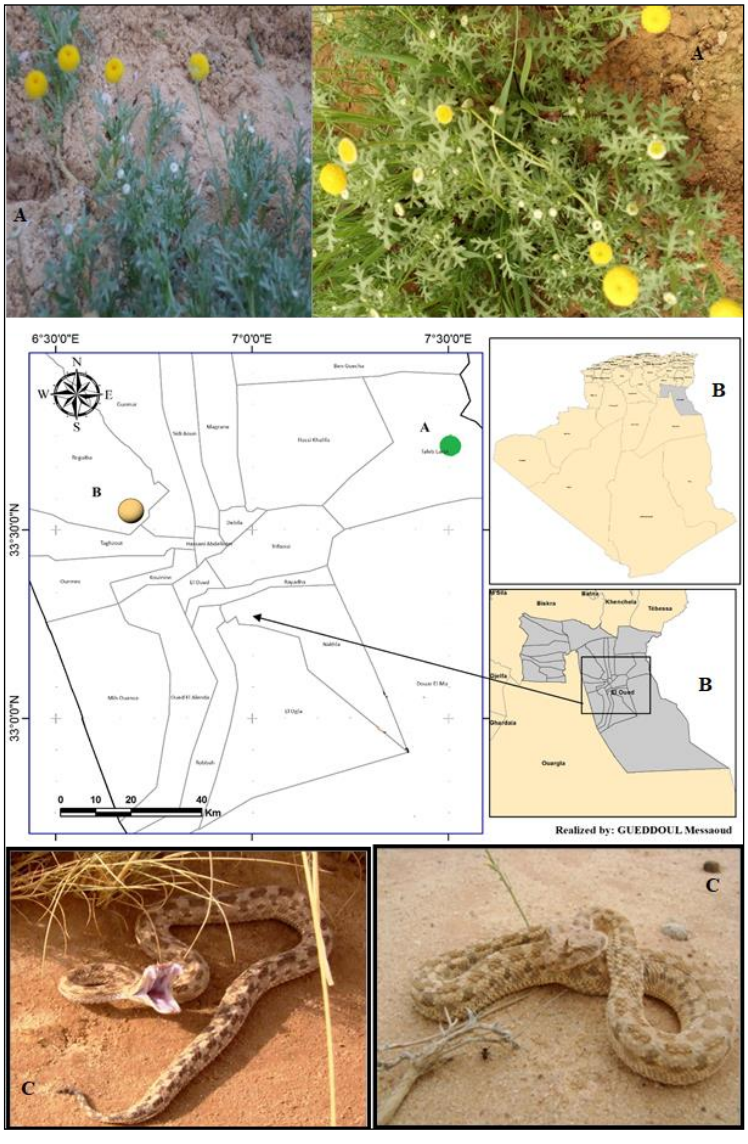


Fig 1: *Matricaria pubescens* (A); geographical location of study area (B), *Cerastes cerastes* (C)

2. Venom Extraction

Cerastes cerastes (Fig. 1C) is measured about 30-60 cm with a max reported length of 85 cm (Schleich *et al*., 1996; Wagner and Wilms 2010; Mallow *et al*., 2003; Mouane, 2020) [50, 55]. Its most remarkable feature was the horned appendages located above the eyes (Nigel & Rob, 2001). The ventral scales from 138 to 186 and 26 to 46 double caudals (Mouane, 2020), 28 to 35 (Le Berre, 1989) and 26 to 37 dorsal scales across the body (Gruber, 1992; Schleich *et al*., 1996) [50]. Snake venom is an amber or whitish-yellow liquid, it is a mixture of complex compositions, secreted for the immobilization and digestion of the prey. It has a viscosity of 1.5 to 2.5 and a density of 1030 to 1050. Their pH varies from 5.5 to 7 (Boquet, 1966).

We extracted the venom of three individuals of this desert horned viper, where the studied specimens were snared, mixed, at the end of September 2017, from El-Oued region exactly in the municipality of Regueba, which their average total length was of 65.8 ± 4.5 cm (Fig. 1A, C). The venom extraction was done manually by hand, by pressing on the head and the tail of the individuals to stimulate the excretion of venom. This operation was performed by Mr. Makkaoui Maamer at the laboratory of biology at the Faculty of Natural and Life Sciences at Echahid Hamma Lakhdar University (Fig. 2).

3. Animal Material

The present experiment was carried out on 16 male of Albino rabbits, provided by Pasteur' Institute in Algiers. Their weight varied between 1.5 to 2 kg. We divided our rabbits into four batches (groups), in reason of four animals/treatment. The first batch was the control (without any treatment), the second one was injected with the venom, the third group was injected with the extract of the selected plant (Matricaria pubescens), and the last group received an injection of aqueous antivenom serum. All treatments were injected intraperitoneally. The doses of the injection were prepared according to the protocol of Laraba *et al*. (2009) [30], considering 25 µg/20g of the rabbit weight for the venom and 100 µl/200g of the rabbit weight for the plant extract. The treated rabbits were sacrificed after 24 hours of injection. For the biochemical analysis, blood samples were taken in tubes without anticoagulant and the serum was obtained by centrifugation of samples for 15 min at 3000 rmp/min (4° C) for the determination of the biochemical parameters: Glycemia, Creatinine, Transaminases (GOT/GPT), urea, cholesterol, triglycerides, fibrinogen, partial thromboplastin time (PTT) and prothrombin time.

Also, for the histological sections, fresh pieces of tissues from different organs (heart, liver, kidneys, brain, lungs, and muscle) were quickly fixed in neutral formalin (10%) and then embedded in paraffin, sectioned (5 m) with a rotary microtome, and stained with hematoxylin and eosin. Then, they were examined under a light microscope with a camera.

Results

The present experimentation allowed us to collect 38 µl of Cerastes cerastes venom where the extract yield of Matricaria pubescens was 11%.

It should be noted that the toxicological study showed that the injection of Cerastes cerastes venom and the aqueous extract of Matricaria pubescens caused behavioral changes characterized by frequent movements in the cages, significant edema in the leg towards the direction injection followed by difficulty in moving, excretions of their excreta.

1. Histological Sections of Treated Rabbits' Organs

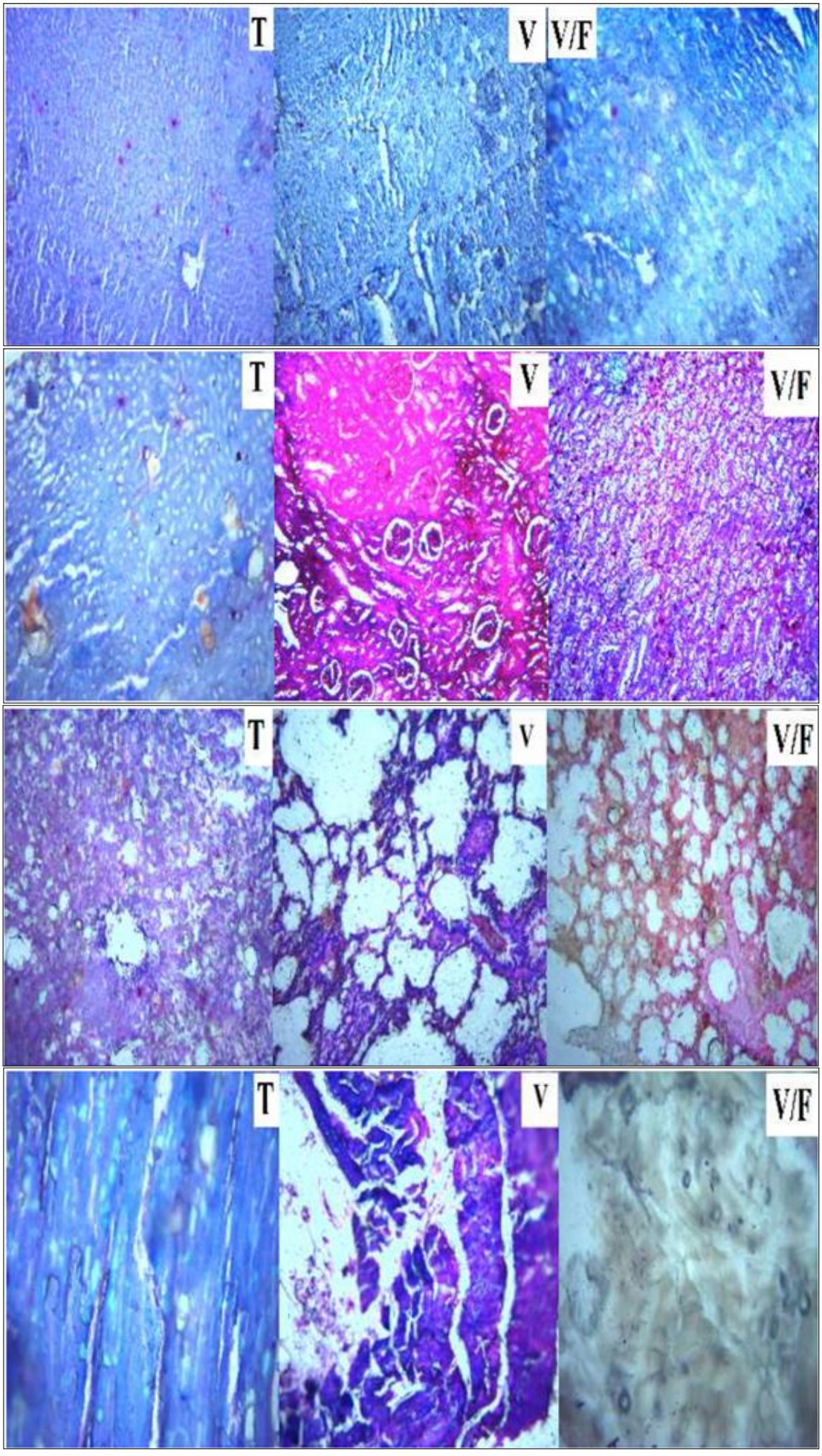
The observation of histological sections from tissues of different organs (liver, kidneys, lungs, muscles, heart, and brain) from the controls allowed us to observe a normal structure in all of their organs (Fig. 2). Unlike the organs of the treated rabbits, firstly, the livers of the 2nd group presented a degenerative modification, tissue damage, and sinusoidal dilations with a cell lesion in the 3rd group (Fig. 2A).

On the other hand, the histological sections of the kidneys showed a necrosis, and a glomerular dilation in the 2nd group and a cellular cytolysis in the 3rd group (Fig. 2B). While the observed histological sections in the lungs showed us an enlargement of the alveoli in the 2nd group, unlike in the rabbits treated by the venom and flavonoids, their alveoli were contracted (Fig. 2C).

Meanwhile, the histological sections of the muscle in the control showed a normal architect (presence of nuclei and muscle fibers). Unlike the muscle of those injected by the venom, was observed the presence of dissociated fibers (Fig. 2D).

According to the histological sections of the hearts of the treated rabbits, the second group showed a dilation at the level of the arteries with contractions at the level of the arteries in rabbits treated with flavonoids (Fig. 2E).

In addition, the histological sections observed in the brain of the control rabbits showed a normal structure while preserving the cell architect with normal vessels. On the other hand, the rabbits of the second group showed a clear prevascular halo around the vessels, while the third group didn't show any halo (Fig. 2F).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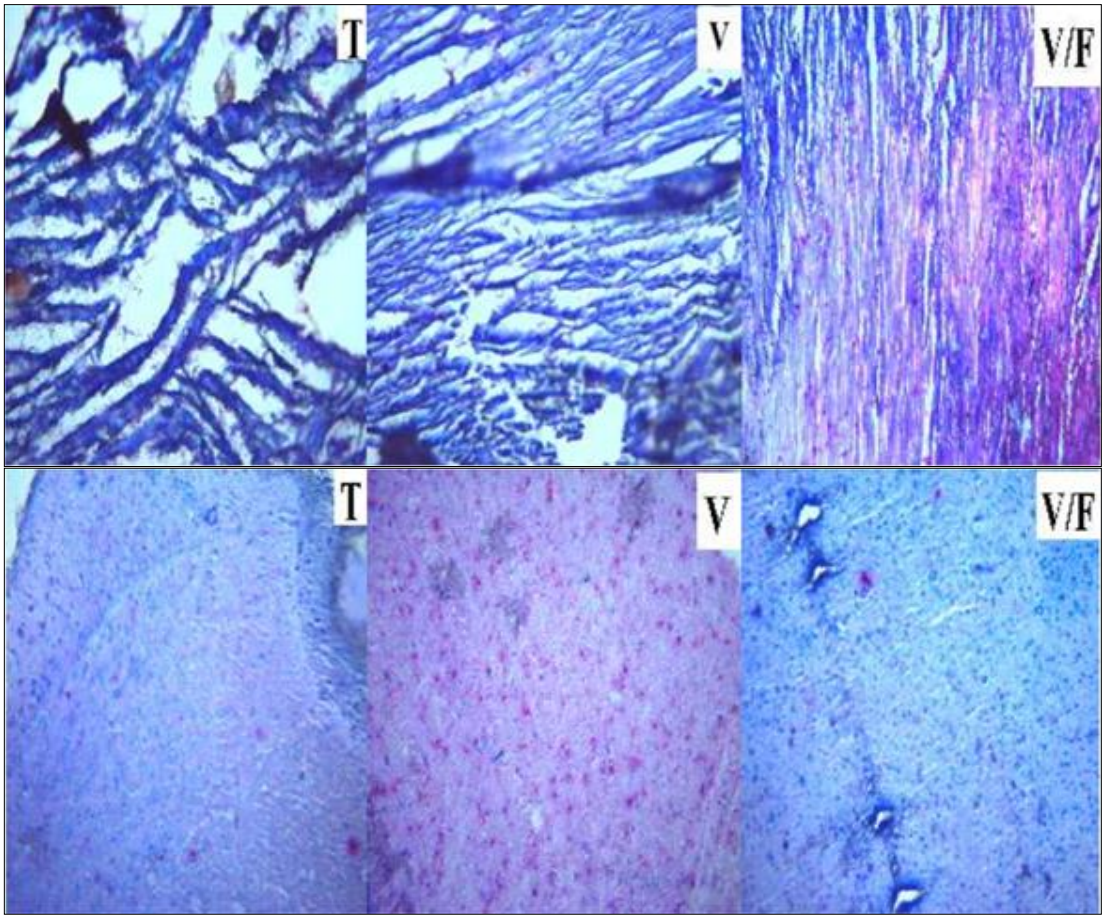

Fig 2: Histological section through the liver (A), kidneys (B), lungs (C), muscles (D), heart (E), and brain (F) of treated rabbits (T: Control; V: Treat with venom; V/F: Treat with venom and plant extract)

2. Effect of Venom and Plant's Extract on Rabbits' Metabolisms

The effect of the different applied treatments, venom and M. pubescens extract, on the metabolism of treated rabbits, was presented in the figure 3 and 4.

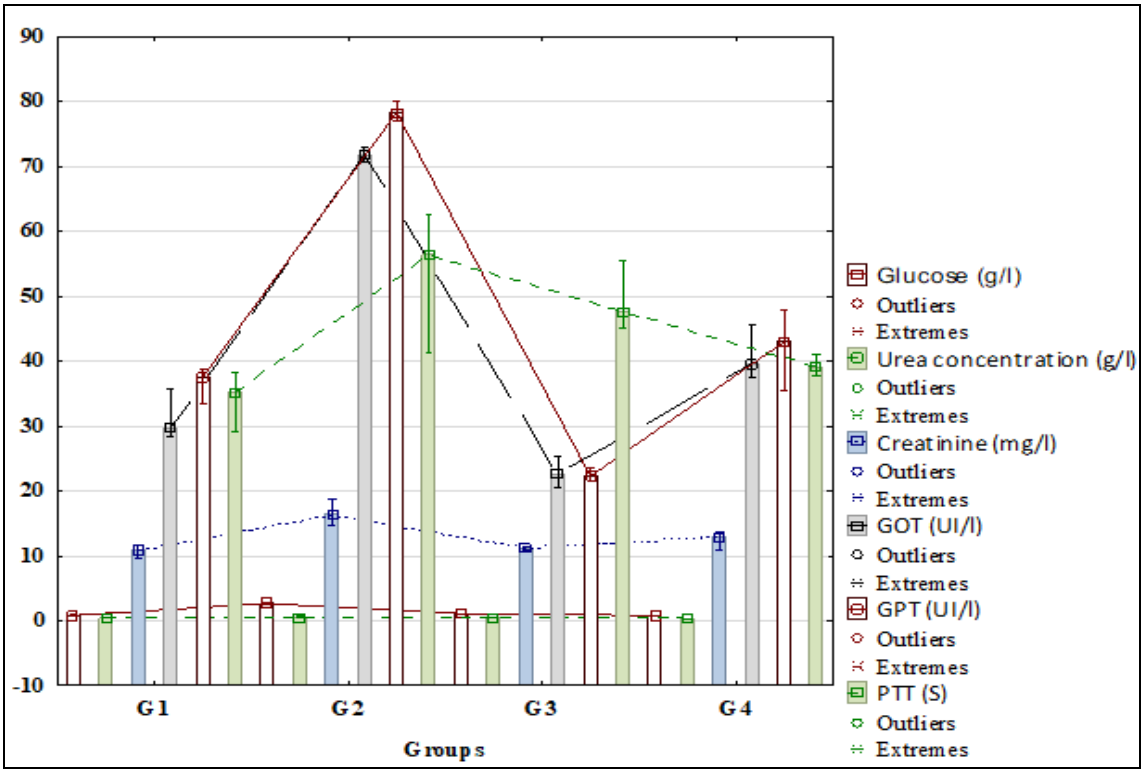

Fig 3: Effect of treatments on the metabolism of rabbits

The present experimentation allowed us to observe that the concentrations of glycemia, urea, and creatinine were superior to those recorded in the control (Fig. 3). It is observed that there was a highly significant increase in blood sugar, in the rabbits injected with the venom compared to the controls with an average of 2.7 ± 0.09 g/l. On the other hand, there was a highly significant decrease in glycemia in rabbits injected with the venom and the plant extract compared to rabbits injected only with the venom. Where the blood sugar of this group varied between 1.03 and 1.15 g / l (avg = 1.1 ± 0.05 g/l) (Fig. 3).

Concerning the activity of TGO and TGP, it was observed a highly significant increase in the rabbits which are injected by the venom with an average of 71.76 ± 0.95 ɥl/l and 78.39 ± 1.29 ɥl/l, respectively, compared to the controls. Unlike, the rabbits of the 3rd group showed a highly significant decrease in these both parameters compared to those of the 1st and 2nd groups (Fig. 3).

For the urea, a significant increase was observed in all rabbits injected with the venom compared to the controls and the 4th group (Fig. 4). Unlike in the rabbits of the 3rd group, a significant decrease was observed (avg = 0.41 ± 0.02 g/l) compared to those injected with the venom (avg = 0.65 ± 0.06 g/l). The statistical analysis showed that there was a very highly significant (p = 0.0000) between all the groups in the increase of glucose, urea concentration, TGO and TGP and a highly significant in the creatinine (p = 0.0002) and the PTT (p = 0.0015) (Fig. 3).

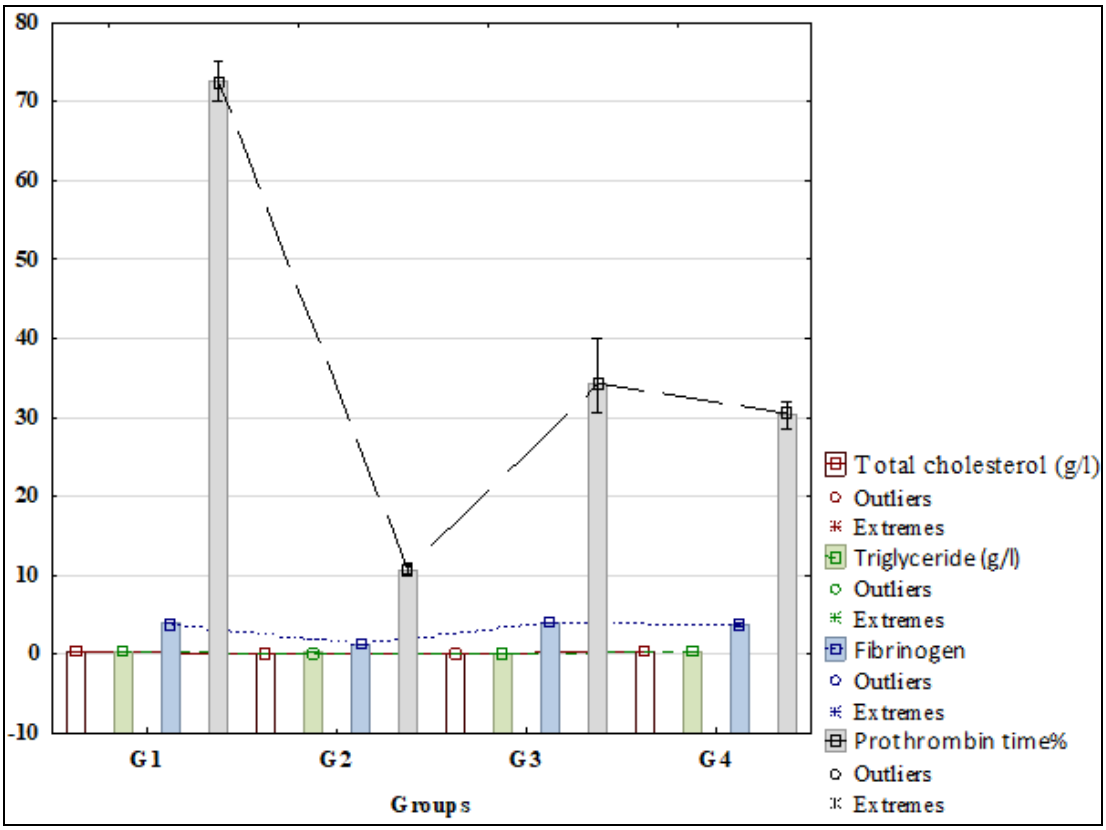

Fig 4: Effect of treatments on the metabolism of rabbits

The figure (5) showed a very highly significant (P = 0.0000) decrease in total cholesterol, in the rabbits injected by the venom as well as the venom and the extract compared to those of the control and the 4th group with 0.055 ± 0.007 g/l and 0.14 ± 0.004 g/l, respectively. Unlike the rabbits injected with the venom and extracts, there was a highly significant increase in total cholesterol, compared to rabbits injected with the venom.

The triglyceride analysis showed a highly significant decrease in the rabbits injected by the venom (avg = 0.23 ± 0.002g / l) as well as those injected by the venom and extracts (avg = 0.16 ± 0.005g / l) compared to the control. Meanwhile, a decrease in triglyceride was observed in the rabbits injected by the venom and the plant extract compared to those injected by venom (Fig. 4). On the other hand, the fibrinogen analysis showed a significant decrease in the rabbits injected by the venom (avg = 1.2 ± 0.086 g/l) compared to the control, group 3 and 4. It should be mentioned that a significant decrease in Prothrombin time between all the rabbits of the second group compared to the control, as well as the 3rd and 4th group (Fig. 4). Statistical data confirmed that there was a very highly significant (p = 0.0000) between all the groups (Fig. 4).

The Relationship between Metabolisms of Treated Rabbits

The figure (5) below presented the relationship between biological parameters of treated rabbits and their concentration effect between each other.

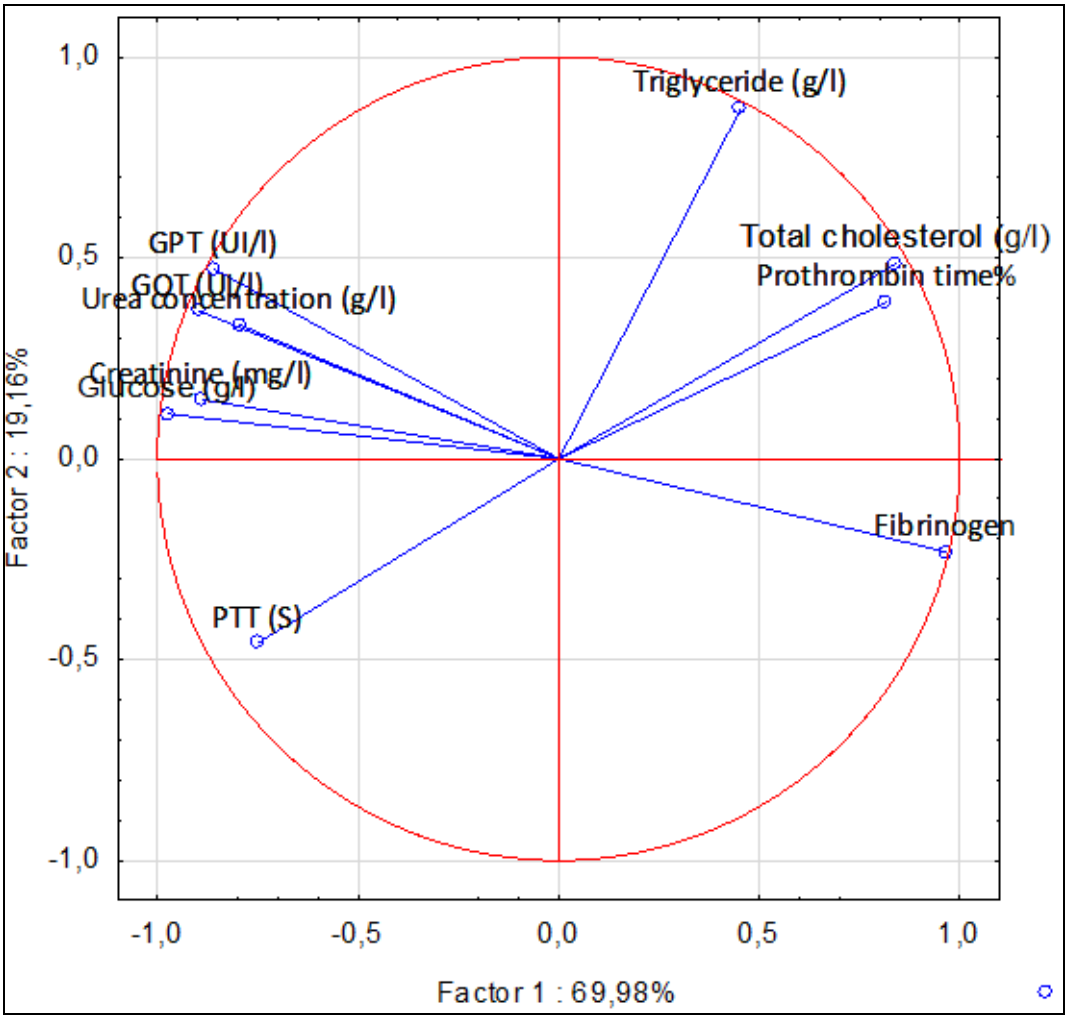

Fig 5: Relationship between biological parameters of treated rabbits

According to the PCA analysis, the biological parameters (cholesterol, triglyceride, fibrinogen, and prothrombin time) were very close, a certain similarity was reflected between them which means that these parameters were influenced by each other. Same thing for the glycemia, urea, creatinine, GOT, GPT, and PTT.

Discussions

The bites of the venomous snakes constitute a medical, social and economic problem for African, low-income rural populations and remote from health centers where the incidence is difficult to know with precision (Bon, 1994). For this, the estimation of 1 million bites in Africa allowed the death of 20.000 and 100.000 person/year. In fact, a large majority of victims first consult traditional therapists because the hospitalization rate is certainly less than 50% (Newman *et al*., 1997) [42]. In total, 1127 species of snake antivenom plants in the world distributed in 176 families where Fabaceae, Asteraceae, Euphorbiaceae, Rubiaceae, Apocynaceae, Lamiaceae, Araceae, Malvaceae, Acanthaceae were the most cited (Felix-Silva *et al*., 2017). That is very important to find safe and effective antivenom compounds from natural sources (Salama *et al*., 2017) [49]. Even antivenom is the only specific treatment available, it does not provide sufficient protection against the venom. In addition, it can stimulate local tissue damage and often hypersensitivity reactions occur (Thwin *et al*., 2010) [53]. Meanwhile, for any bite, biological assessment and clinical signs are necessary to allow a decision on the degree of envenomation (Lamb *et al*., 2017) [29].

According to the obtained results from the present study, we recorded a hyperglycemia in all the rabbits injected by the venom supplied to those of the control. Our results could be justified by the explanation of Valenta *et al*. (2016) [54], where they noted that the venom of Cerastes cerastes deteriorates the metabolisms of the pancreas and the liver accompanied by changes in plasma insulin and glucagon concentration. Also, Mohamed *et al*. (1963; 1972) [27, 38], confirmed that snake venom causes hyperglycemia in experimental animals. This increase could be explained by the effect of venom on the glycemic hormones, either by structural modification of the hormones (inactive) (Jaen & Bruno, 2001) [26]; or by their influence on the pancreatic cells responsible for the secretion of hormones.

On the other hand, a hyper-creatinine was recorded by the increase of urea concentration and kaolin partial thromboplastin time with a decrease in fibrinogen and prothrombin blood levels specially in the 2nd group of rabbits compared to controls. Several studies confirmed our results where Mebs and Goyffon (2006) noted that the envenomation was characterized by an increased inflammatory cytokine, thrombocytopenia, afibrinogenemia

with a decrease in prothrombin level and an increase in the activated partial thromboplastin time. Also, Chippaux (2002) [16] recorded an increased plasma creatinine which was a sign of renal failure. In addition, a highly significant increase in the activity of GOT and GPT, in the rabbits of the second group, was recorded. Similar results confirmed the obtained results in this experiment, where the levels of these enzymes (GPT and GOT) have been increased when they were injected with the venoms of Cerastes cerates (Abib, 2002) [1], Androctonus australis hector (Bessalem *et al*., 2003) [10], and Vipera lebetina (Amrane, 2002) [3]. These results could be explained by the act of the venom on the hepatic cells and the kidney cells by bursting the cell membrane which caused the secretion of the amount of GOT and GPT enzymes increased in the blood (Rollard *et al*., 2015; Valenta *et al*., 2016) [47, 54].

Furthermore, the results showed us that the treatment by peritoneal injection of the aqueous extract of Matricaria pubescens, presented an obvious effect against the activity of the venom by a relative normalization of the biochemical parameters (neutralizing the effects of the venom). A large number of ethnobotanical studies have reported the neutralizing properties of snake venoms from several medicinal plants (Gomes *et al*., 2010; Felix-Silva *et al*., 2017, Salama, *et al*., 2017) [23, 49]. Some herbal remedies produce compounds effective against snake envenomation such as tannins, alkaloids, acids, xanthenes, quinoids, glycoproteins, phenols, steroids, glycosides, enzymes, peptides, pigments, pterocarpans, terenoids which neutralize various enzymes and toxins in venoms (Gomes *et al*., 2010; Barma *et al*., 2014) [23, 6]. According to Gherboudj (2014), who worked on the extraction of M. pubescens allowed the isolation and the identification of 11 flavonoids where Apigenin and Quercetin have an anti-inflammatory property and reduce the level of bad cholesterol (Prouillet, 2004; Ghedira, 2005; Si *et al*., 2009; Namsi *et al*., 2019) [45, 21, 52, 41] and Luteoline gent preventive of inflammation, as a compound helping carbohydrate metabolism and as a regulator of the immune system and anti-inflammatory (Moldovan, 2014) [39].

Confirmation of the biochemical results was provided by the histopathological study, which showed considerable histological disorders and changes in tissue structures of various organs (brain, heart, liver, muscle, kidney and lung) in envenomed rabbits. Unlike, the histological sections of the organs of envenomed rabbits treated with the aqueous extract of the tested plant (M. pubescens) where they showed a less significant tissue alterations. According to Chippaux (2002) [16], the venom of the Viperidae was rich in proteolytic enzymes which lead the destruction of cellular and/or interstitial structures without rapid management, necrosis gradually appears at the bite level.

Author Contributions

MA, MNE and MR are responsible for the conceptualization, supervision; validation of roles/writing-original draft. MR designed, provided statistical analysis and academic input in writing the manuscript and edited the various drafts. DN and BZ have done the methodology.

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