RESEARCH ARTICLE

Preliminary *In-vivo* study on rats to enhance recognizing the use of *Anacyclus clavatus* as traditional diet by the local population from Tizi-Ouzou

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This study aimed at evaluating some biochemical and pharmacological properties of the aerial parts (stems and leaf) powder of *Anacyclus clavatus* conventionally used by the local population of Tizi-Ouzou (northern Algeria). 18 male Albino-Wistar rats were assigned to three equal groups: Control, those inflamed with black pepper solution and treated with the aerial parts water extract powder of *A. clavatus*, and those treated with the aerial parts water extract of *A. clavatus*. Thus, the assessments of some physicochemical and functional characteristics of tablets have been studied in three different ways imitating certain physiological conditions by applying the same methods as those used in the pharmaceutical industry. Feeding rats with 10 g/kg body weight with the aerial parts water extract for 2-4 weeks shows a significant reduction in blood glucose levels, prevents elevation of lipid serum level and gastric inflammation, as well as the protection against body weight loss. The plant reveals the presence of multiple inflammatory cells in the liver and the lungs. The analysis results presented important chemical constituents such as (Na, Mg, Fe, and cu), bioactive substances (polyphenols, flavonids, tannins), and richness in essential fatty acids. The greatest swelling yield of tablets has been observed in distilled water and phosphate buffer pH 6.8 (100, 99%), without erosion. The grain size ranging from 275 to 316 µm offers very interesting properties in terms of compacting, handling, and speed releasing in different mediums. This approach encourages the use of the powder for health applications by optimizing the dose with a therapeutic effect.

Key words:*Anacyclus clavatus;* Anti-inflammatory; Hematological properties; Nutrition; Tablets

INTRODUCTION

Anacyclus clavatus is a common perennial plant belonging to the Asteraceae family. It has been known since the antiquity for its medicinal properties. This Mediterranean species is very common throughout Algeria. It has a very short vegetative cycle blooming between April and June. Nevertheless, it is spontaneous along the roadsides, removed land, dry nitrophilous grassland, up to 1,600 meters above the sea. It is an aromatic plant, and it is characterized by a specific odor.

This species has undergone several chemical investigations, indicating the presence of many types of secondary metabolites, including triterpenes, steroids, coumarins, lignans, polyacetylenes (alkamides) and flavonoids.

The information gathered during our own ethno-botanical survey carried out during February and March of the year 2016 in several sites in Algeria constitutes an inventory which contributes, on the one hand, to the knowledge of the medicinal flora and, on the other hand, to a safeguard of the know-how of local populations.

The area named BeniYenni (in Berber: AthYenni) is a rural 'county' (Daira in Arabic) located in the northern side of the Djurdjura mountain, some 35 km southeast of the city of Tizi-Ouzou. The current plant species is overused by people aged 40-70 (46%). Its leaves and stems are the most used with the highest percentages of use (86% and 68%, respectively).

This species is known by several names, named "walmam" specific to the region of Bouira, "chib el hart" on the side of the Ouadias and "Tazdelt" at Larebaa NathIrathen.

The inhabitants of these areas use its leaves and stems to prepare couscous, a national traditional dish, in order to treat gastric problems and hemorrhoids.

In other areas, such as Oum El Bouaghi and Djelfa, the plant is known as "chicken feet". What is particular with the inhabitants of these two regions is that they limit themselves to the use of its flowers for a preparation in infusion, and this is recommended against anxiety. As for the stems, they are intended to soothe stomach ulcer attacks.

This information is well in accordance with a number of scientists, who declared that its leaves and stems were used in the diet in salads or compotes and as digestive teas [1-3] also against gastric ulcers [4]. It is also used in the form of an anti-inflammatory plaster [2]. The roots have been used to treat various pathologies; they have been thought to be particularly effective against skin infections, especially fungal diseases.

To our knowledge, no scientific work has been conducted so far to study the impact of the powder obtained from this plant in terms of its physiological functions as well as its pharmacological properties. It is important to ensure about the safety of a plant and know the conditions under which it can be used. For this reason, we considered it useful to shed light on the major anti-inflammatory activities on rats as defined by conventional pharmacopeia in the area concerned in the present study and to highlight some pharmacological properties of the tablets by applying the same methods as those used in the pharmaceutical industry. Thus, the determination of certain physicochemical properties of tablets (friability, swelling yield, release time) were studied in three different ways imitating certain physiological conditions (distilled water and phosphate saline solution at pH 6.8 and 0.1 N HCl).

The analysis made on the chemical composition of essential oils reveals the presence of 46 compounds, including chrysanthenyl (81.2%), acetate (12.3%), thujone (9.8%) and chrysanthenone (8.2%). These are the major compounds characterizing the aerial parts (leaves, stems and flowers) of this plant of Tunisian origin [5]. Comparing these results with those characterizing the plant of Algerian origin, we have identified more than

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106 volatile compounds, of which Germacrene D (16.84%) constitutes the main volatile component extracted from the leaves and stems whereas β-thuyone (11.16%) is the main constituent of flowers [6].

It should be noted that several compounds have very interesting therapeutic effects, in particular α-cadinol, a compound with a selective toxic effect against human cells of adenocarcinoma of the colon [7]. β-elemene is used as an anti-tumor drug [8]. As for Rimbau [9], they have highlighted the anti-inflammatory activity of *A. clavatus* extracts against cytokine synthesis.

Other compounds, such as 1,8-cineole, terpinene-4-ol and α-terpinene are distinguished by their high insecticidal activity [10]. Besides, the studies conducted Hammami et al. [5] have shown that the essential oils of *A. clavatus* have an antibacterial activity towards *Pseudomonas aeruginosa,* and a greater antifungal activity against *Candida albicans, Candida glabrata, Candida krusei* and *Candida parapsilosis.*

The leaves and stems are very rich in phenolic compounds (polyphenols, flavonoids and tannins). In herbal medicine, even if some indications are common to several classes (the vasculo-protective properties are, for example, as well attributed to flavonoids as to anthocyanins, tannins and other coumarins), each chemical class seems to be used for specific benefits [11].

It should be noted that this plant is a variable source of phenolic and aromatic compounds depending on several factors (climate, stage of maturation, physiological stress, etc.). It should also be noted that the consumption of this plant is limited to the traditional scale and stimulated by the population of the region of Tizi-Ouzou to treat gastric disorders. Therefore, it is recommended that clinical studies be conducted to accurately detect the impact of this plant on consumer health (dose-effect).

MATERIAL AND METHOD

Material

Vegetal material: The aerial parts (leaf and stems) of *Anacyclus clavatus* from the area of BeniYenni in the Wilaya of Tizi-Ouzou were used in this study. They were harvested in the morning as recommended by Nogaret-Ehrhat, (2008) in the period running between February and March 2016.

Animals: Adult albinoWistar laboratory rats, «Rattusnorvegicus», (24 males) weighing (192-205 g), aged two to three months, were tested in this study. The rats were brought from the Laboratory of Pasteur Institute located in Algiers.

Methods

Manufacturing of powder: Just after being harvested, the aerial parts (leaf and stems) of *A. clavatus* were dried at ambient air and grinded using an electric disintegrator. The dried powder of the aerial parts of *A. clavatus* (4% d.b) was sieved between 200 and 350 µm using an adequate sieving system (ECHARDT 5657 HAAN, Germany) before making the tablets.

***In-vivo* study:** This part of the research work was carried out at the Laboratory of Pasteur Institute located in Algiers during four weeks (May to June 2016) and the experimental protocols were approved by the local Ethical Committee of the same Institute.

Experiments were carried out in adult Wistar rats (18 male) weighing 192-205 g, which were fed with a standard diet and water.

The rats were divided into three groups: control, group inflamed with black pepper water solution and treated with the aerial parts water extract of *A. clavatus,* and the third group only with the aerial parts water extract of *A. clavatus.*

The aerial parts water extract of *A. clavatus* in a dose of 10 g/Kg body weight dissolved in sterile water, was given orally to two groups of animals (n=12) daily for 4 weeks. The last group (n=6), representing the control group, was daily fed orally, with sterile water. All the animals tested were fed with 30 g of standard diet. The litter is renewed three times a week.

Preparation of ulcer gastric animals: Black pepper powder was dissolved in sterile water immediately before use. Then it was injected in a dose of 1 g/Kg body weight. After 24 hours, the animals were treated with the same aerial parts water extract of *A. clavatus* in a dose of 10% immediately prepared.

At the end of the study, the animals were sacrificed under deep etheranesthesia.

- Collection of blood: Before feeding the animals, the body weight had been recorded and blood samples by an enzymatic method, using COBAS INTEGRA HDL CHLOSTEROL plus 2nd generation (HOL.O). The principal of the method used is described by [12].
- Determination of serum triglyceride: The serum triglyceride was estimated by means of an enzymatic method, using COBAS INTEGRA TRIGLYCERIDE Kite.

Histology procedures

The Histo-pathological examination of the organs was carried out at the Laboratory of Anatomy and Veterinary Cytology and Pathology at the Pasteur Institute of Algiers.

The organs were fixed in 4% neutral buffered formalin, paraffin embedded, cut in 5 µm section and stained with Hematoxylin-eosin according to the procedure described in the experimental protocols of the same Institute.

Compression of powder

Flat-faced tablets (diameter=40 mm) were prepared through direct compression using a tablet-press (HERAZOG) in the Laboratory of Research Unit, Materials, Processes & Environment(UR-MPE) of the University of Boumerdes.

An optimal compression force in terms of suitable friability was processed at 200 KN and dwell time of about 25 seconds. Below this value, the tablets were very friable, and above this value, the tablets were excessively hard.

Physico-chemical and functional characteristics of tablets: The assessments of the physicochemical and functional characteristics of the aerial parts (leaf and stems) of *A. clavatus* powder and tablets were monitored between May and June 2016. These assessments were all done in the Saidal laboratory (Dar-El-Beida, Algiers) in conformity with the European Pharmacopoeia [13].

The friability (%) was determined by means of a friability tester (ERWEKA TA 40, Germany). The device was loaded with 10 tablets and then turned on for 4 minutes at 25 rpm (rotations per minutes).The friability (F) was calculated as:

$$F\% = \frac{\left(P_0 - P_1\right)}{P_0}$$

Where F is friability or weight loss (%, w/w), Po and P1 are the initial and final values of the tablet weight (g).This test was applied without repetition.

The swelling yield was evaluated through liquid uptake by the tablet placed in 3 different mediums heated at 37 ± 0.5°C (distilled water, HCl 0.1N and phosphate buffer pH 6.8).The erosion test immediately following the swelling consisted in the determination of the dried weight of the wet tablets by drying them at 80°C during 24H.

Bioactive substances: The richness of this plant in bioactive substances offers it anti-inflammatory properties, such as Total Polyphenol Content (TPC), flavonoids and chlorophyll, as estimated in this study.

Determination of TPC-The experiment was conducted with the Folin-Ciocalteu method modified by [14]. The absorbance at 710 nm was

measured by means of a spectrophotometer (EV 9200, Germany). The regression equation of calibration was obtained with various concentrations of the Gallic acid standard. Three replicates per treatment were used to calculate the TPC value, expressed by µg of Gallic acid equivalent per mg of dry basis (µg GAE /mg db).

The flavonoids were measured in a Colorimetric way at 430 nm on the basis of the method suggested by [15], which consists in mixing and dissolving 1 ml of the plant extract with 1 ml ALCl3 (2%). The regression equation of calibration was obtained with various concentrations of the Quercetin standard and expressed by µg of Quercetin Equivalent per mg of dry basis (µgQE/mg db).

Total chlorophyll: The optical density of the aerial parts of *A. clavatus* extract was determined to 660 nm and to 642.5 nm by means of a spectrophotometer (EV 9200, Germany) according to the procedure described (Ramesh, 2000).

Lipid content estimation: This was determined on the basis of the hexane extraction method (French Official Journal, 2008). The fatty acids were determined by Gas-Chromatography GC using Chrompack CP 9002. Methyl esters were formed by trans-esterification in a methanolic solution of potassium hydroxide as an intermediate phase prior to saponification following the ISO method (2000).

Salt quantification was achieved through Atomic Absorption Spectroscopy, using an Atomizer (VARIAN AA 240, Australia). This method relies on the dissolution of 1 g of ashes with 5 ml of HCL acid (0.5 N) (Adrian, et al.1995).

The Morphological examination of eroded tablets was performed after their immersion in different liquid mediums using a digital camera equipped with a 14.3xlens (Fuji, China).

STATISTICAL ANALYSIS

The results are presented as means ± S.E.M. A Student's paired t-test was performed to calculate the statistical significance between mean values of different levels of significance. The values are considered statistically significant when $P<0.05$. The statistical analyses were performed by means of an Origin Pro 9.0 Software.

RESULTS

Results of *in-vivo* study

Morphological examination: The microscopic examination of the stomach, intestine, liver and lungs of negative control, revealed no lesions.

Histo-pathological findings

- Batch inflamed and fed with plant extract: The histo-pathological examination revealed a multifocal, slight and sub-acute peri-portal and intra parenchymal inflammatory infiltration in the liver specimens.
- The microscopic examination also revealed a Kupffer cells hyperplasia (Figure 1a) and a multifocal slight and sub-acute inflammatory infiltration in the chorionic portions of the intestine specimens (Figure 1b).
- Batch Fed with plant extract: The histo-pathological examination of the lungs revealed a diffuse, marked inter alveolar and peri-bronchial inflammatory infiltration (Figure 2).
- Blood parameters : The determined results indicated that the plant extract exhibits the following effects:
- An anti-inflammatory activity against gastric ulcer.
- More protection against body weight loss ($P<0.02$).
- Maximum reduction in the blood glucose level ($P<0.001$) (hypoglycemic activity).
- A reduction in the elevated serum cholesterol level and cholesterol HDL, ($P<0.001$, $P<0.01$, respectively).
- An increase in the triglyceride level ($P<0.001$).
- Multiple inflammatory cells of the liver and the lungs.

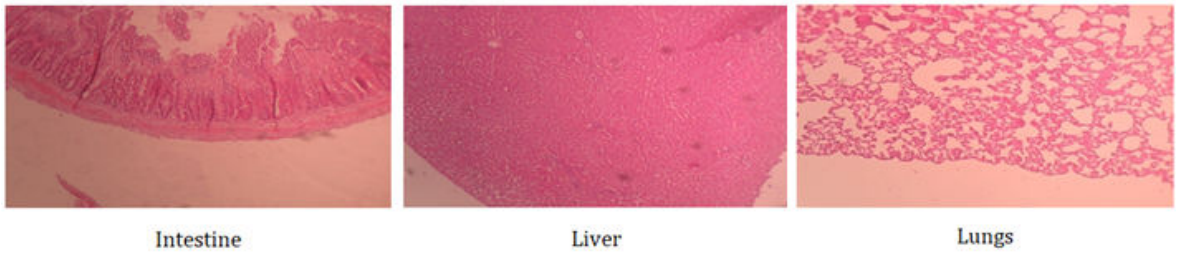

Figure 1 Histo-pathological examination of batch inflamed and fed with the aerial parts (leaf and stems) water extract of A. clavatus a) Liver: subacute periportal and intra parenchymal inflammatory infiltration. Hematoxylin eosin. Gx4 b) Intestine: multifocal slight and sub-acute inflammatory infiltration in chorionic portions. Hematoxylin eosin. Gx4 c) Lungs: multifocal, slight, sub-acute inter alveolar and peri bronchial inflammatory infiltration. Hematoxylin eosin. GX4.

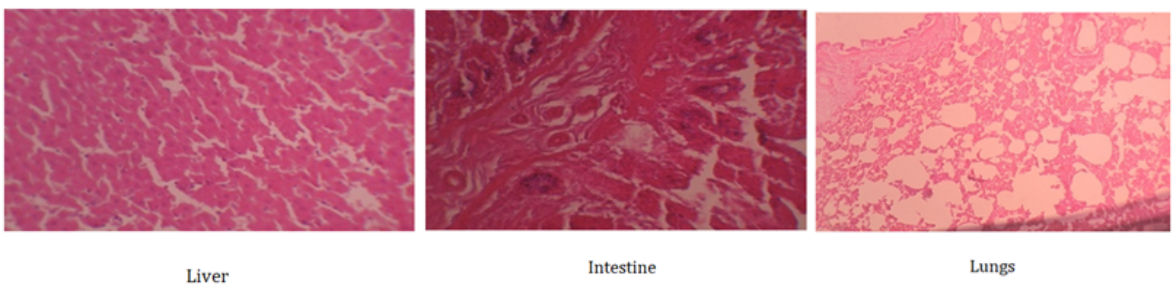

Figure 2 Histo-pathological examination of a group fed with the aerial parts (leaf and stems) water extract of *A. clavatus* a) Liver: several kipper cells in the cinosides multiple inflammatory cells. Hematoxylin eosin. Gx4 b) Intestine: no lesions revealed. Hematoxylin eosin. Gx4 c) Lungs: Diffuse, marked inter alveolar and peri-bronchial inflammatory infiltration. Hematoxylineosin. GX4.

All in all, it should be noted that no cases of mortality have been recorded throughout the study period even after 1 month of treatment break down (Table 1).

Table 1 Percentage of changes of some blood parameters in rats treated daily for 2-4 weeks with 10 g/Kg body weight with the aerial part water extract of *A. clavatus*.

Treatt time	Body weight (g)			Blood glucose (g/l)			Total Cholesterol (g/l)			Triglyceride (g/l)			CholosterolHDL (g/l)		
	C	I -T	T	C	I -T	T	C	I -T	T	C	I -T	T	C	I-T	T
BeforeTreatment	247±6	224***±10	238±13	0.75±0.02	0.73± 0.03	0.72±0.12	0.83±0.02	0.76*** ± 0.04	0.75****± 0.03	0.61 ± 0.02	0.56**± 0.03	0.54±0.11	0.6± 0.02	0.6± 0.04	0.55± 0.11
2 weeks	235±9	202***±19	221*±7	0.68±0.05	0.81****±0.002	0.62*±0.004	0.732±0.02	0.73±0.03	0.62****±0.01	0.59 ± 0.01	0.64***±0.02	0.75****±0.025	0.39±0.071	0.53***±0.005	0.45±0.003
% of changes 2 week after	-4.81	-9.83	-7.18	-9.3	19.95	-13.88	-11.8	-3.94	-17.33	-3.27	14.28	38.88	-3.5	-11.66	-18.18
4 weeks	234.96 ±17.441	231.64± 6.12	263.24** ± 11.23	0.640± 0.19	0.55± 0.14	0.52±0.29	0.58 ±0.002	0.7**** ± 0.011	0.61****± 0.007	0.58 ± 0.01	0.65***± 0.033	0.91****± 0.001	0.37±0.056	0.28***± 0.023	0.3± 0.04
% of changes after 4 weeks	-4.81	3.33	10.52	-14.66	-24.65	-27.77	-30.12	-7.89	-18.66	-4.91	16.07	68.51	-38.33	-53.33	-45.45
Standards		-			0.75-1.10			1.3-2.2			0.5-1.6			0.35-0.55	

C: Control; I-T: inflamed+Treated with the aerial parts water extract of *A. clavatus*, T : treated with the aerial parts water extract of *A. clavatus*
Significantly different from control by *P<0.05, **P<0.02, ***P<0.01, ****P<0.001, Student's t-test.

Results of some physical-chemical parameters of tablets

The physical properties of the tablets obtained from the aerial parts (leaf and stems) of *A. clavatus* powder are displayed in Table 2. After the comparison of the size of the prepared tablets, it was confirmed that all of them were equivalent, which can facilitate the analysis of their physical properties.

Table 2 Physicochemical properties of the aerial parts of *A. clavatus* Tablets (n=10).

Parameters	Tablets	Standards (EP, 2015)
-	8±0.6	>0.650
Weight (g)	40.57±0.03	Variable
Diameter (mm)	8.04±0.43	Variable
Thickness (mm)	0.607	>1
Friability (%)	7.610±0.373	>5
Moisture (%db)	82.06±2.78	-
Total Phenolic Compounds (µg EAG/mg dry basis)	-	-
Total Flavonoids	16.56±1.97	-
(µg Quercetin/mg dry basis)	-	-
Chlorophyll (mg/g db)	1,71 ±0,008	-
Time of release (mn) in :Distilled water	-	-
HCl 0.1N	13	<15
Phosphate buffer solution pH 6.8	13	<15
-	13	<15

In our case, the tablets had a higher size in comparison with drogue tablets, and this difference is related to the availability of the type of punch used.

The greatest swelling yield was observed in distilled water and phosphate buffer pH 6.8 (100, 99%), respectively without erosion. The tablets presented a tough texture after having undergone a continual water uptake.

The tablets disintegrate in an acid solution, which allows a lower swelling yield (46%), and this was confirmed by the morphological examination of the tablets during 20min (Figure 3). Our results are consistent with those reported by [16], which showed that Spirulina tablets uptake water without erosion. Indeed, the tablets are characterized by a high level of moisture, owing to their hydroscopicity.

Comparing these results to those reached by other research works, it is important to note that the water retention was related not only to the nature of the mediums used but also to the nature of the compounds that constitute the tablets. We can note some richness in tannins and mucilage.

In fact, an intrinsic difficulty with food powders was their agglomeration and compaction during storage, which is undesirable from the point of view of handling [17], whereas this disadvantage becomes an advantage when it comes to obtain tablets.

Anyway, tablets were characterized by a similar release time in the different mediums. Regardless of the case, the release time found in the present work in the distilled water, HCl 0.1N and the Phosphate Buffer solution pH 6.8 agree with the standard results (EP, 2015). This result matches those of other authors who worked on tablets with different compositions 4.65 min (tablets from dried dates) [6]. It is worth to simulate that *A.clavatus* tablets disintegrate in gastric mediums which have the same pH as an HCl solution.

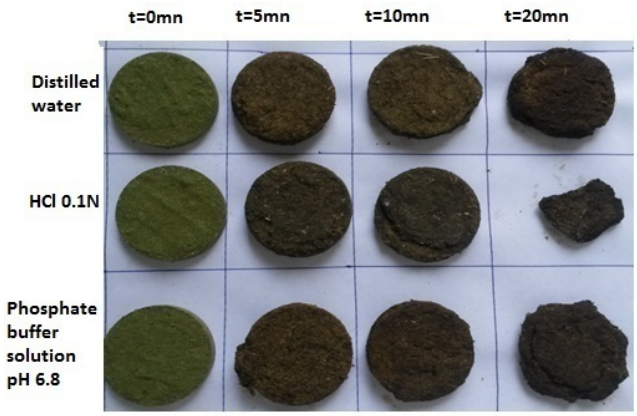

Figure 3 Physical aspect of the eroded *A.clavatus* tablets after immersion in different mediums.

DISCUSSION

The results attained in the present study show that the plant extract protected animals from massive body weight loss, when given orally and daily for 2-4 weeks, in comparison to the untreated group. The ability of the plant extract to protect body weight loss would be due to its ability to reduce the level of serum lipids, in addition to its hypoglycemic effect.

The significant and consistent hypoglycemic effect of *A. clavatus* in rats within 2-4 weeks indicates that the plant extract has a major impact on the glucose utilization by peripheral tissues.

Enough evidence has been accumulated in this study to show that the anti-inflammatory activity of this plant is attributed to its richness in bioactive substances. In fact, the aerial part (leaves and stems) contain flavonoids (cathechin, quercetin, benzoic acid), tannins, gallic tannins and alkaloids), but in different proportions. This richness in these compounds gives it important pharmacological properties, hence their use in traditional medicine.

On the other hand, this plant is devoid of anthocyanins, leuco anthocyanin, saponosides and glycosides. Similar findings were already reported for the same metabolite from *A.clavatus* by Aliboudhar [6].

In comparison with the results of *Anacyclus pyrethrum* studied by [18], we may say that those attained in the present study have revealed that the presence of tannins, alkaloids, flavonoids and saponosides in the species. Moreover, the anti-inflammatory activity is also attributed to the richness of essential fatty acids identified at the most important variable proportions palmitic acid (18.63%), oleic acid (11.14%), linoleic acid (20.31%), and linolenic acid (33.83%).

It is noticed that olive oil has always been recommended for its anti-inflammatory and healing properties. The plant studied contains a high linolenic acid content compared to that found in olive oil in this type of acid (1.01%). Similarly, the palmitic acid and linoleic acid contents are comparable to the olive oil composition (17.06% and 18.54%) [6].

Furthermore, the optimal effectiveness of an extract cannot result from one active constituent, but rather with the combined action (synergy) of various constituents [19].

On the other hand, the study gave a clear view that the aerial parts (leaf and stems) water extract of *A. clavatus* has a significant action on the elevation in the level of triglycerides. High triglyceride levels are associated with a high risk of severe atherosclerosis. In general, elevated triglyceride levels may be either hereditary or subsequent to disorders such as diabetes, nephrosis, biliary obstruction, and metabolic or endocrine disorders.

To our knowledge, this is the first study in which a follow-up is made of the changes in the liver of the positive control. Besides, the batch treated with the plant reveals the presence of several kupper cells in the cinosides-more than usual-as well as the presence of multiple inflammatory cells. This can be explained by the very high dose administered to the rats. Indeed, this dose can induce a hepato-toxic effect that is due to the absorption of the plant by the liver without being metabolized (doses / receptors effect). The latter may cause hepatic insufficiency (liver disease).

The inter alveolar and peri-bronchial inflammatory infiltration is due to the higher availability of aromatic compounds (quercetin, cathechin and benzoic acid).

To counter the effects of the dose we have tested, it is interesting to carry out further studies by optimizing the dose with a therapeutic effect.

In terms of mineral composition, this species is characterized by high levels of essential metals for the organism, such as sodium, magnesium and oligo minerals (Iron, Zinc and Copper) (Table 3). In terms of toxicity, the plant in question does not contain toxic elements such as Cd and Cr. These results are similar to those reported by Bouriche after the analysis of the same species originating from the area of Setif (East of Algeria) [20]. Sometimes, we can find differences in the mineral composition depending on the microclimate, the composition of culture and the species itself; this makes it possible to consider a variety of formulations.

All in all, from the pharmacological point of view, it may be interesting to characterize the plant extract to recommend it for people who suffer from anemia.

However, the physicochemical properties of the powder, such as the bioactive substances, were much more related to the drying process and attributed to the granular texture. In our case, the size of the aerial parts (leaf and stems) of *A. clavatus* powder (ranging from 275 µm to 316 µm) offers very interesting properties in terms of compacting and handling, and it has allowed a very interesting speed of release in the different mediums. This is confirmed by the friability value of the performed tablets. This results in a better accessibility in terms of water/powder interaction (porosity and capillary action).

Table 3 Chemical Composition of the aerial parts of *A. clavatus* powder (mg/kg db) (n=3).

m	Na	Mg	Zn	Fe	Cu	Mn	Ni	K	Cr	Cd	Pb
Quantity	52208 ± 34.1	15096.3 ± 55.23	507.85±0.22	5406.5±12.24	105.2± 22.01	354 ± 5.34	6.2±0.2	<0.02	<0.02	<0.02	<0.02

CONCLUSION

The data obtained in the present study show that the aerial parts (leaf and stems) of *A.clavatus* used as traditional diet by the local population from Tizi-Ouzou has a highly functional quality thanks to its biochemical composition and pharmacological properties. The effects of the water extract of the aerial parts of *A.clavatus* on rats are numerous. First, it not only causes the total disappearance of inflammatory cells (gastric ulcer) with its anti-inflammatory activity, but it also reduces cholesterol levels and cholesterol HDL. Second, thanks to its hypoglycaemic activity it allows the reduction of glucose levels in blood. Third, its consumption engenders an increase in triglyceride levels. Finally, it eliminates inflammatory cells in the lungs and the liver. In sum, our suggestion is that further studies should be conducted in the future in order to optimize the dose with therapeutic effects.

Authors' Contribution

B D.A: Proposal of the research topic, designed the study, did the analysis and wrote the article,

Y.B: Participate on carrying out Histo-pathological examination,

I.M, C.B and A.K: Were involved in overall planning, supervision and critical revision,

B.A: Participate on carrying out statistical study,

B.M, B.A, AS.A and M.L: Participate on carrying out experiments.

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