WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 1405-1419 Research Article ISSN 2278 – 4357

ACUTE TOXICITY AND ANTI-INFLAMMATORY ACTIVITY OF *ASPHODELUS MICROCARPUS*

Hanae Hosni*, Fatima Zahrae Bellaoui, Amina Bounihi, Yahia Cherrah and Katim Alaoui

Research Team on Toxicology and Pharmacodynamics of Aromatic and Medicinal Plants ERTP. PAM, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V in Rabat, Morocco.

Article Received on
21 Feb. 2019,

Revised on 11 March 2019,
Accepted on 01 April 2019

DOI: 10.20959/wjpps20194-13470

***Corresponding Author**
Dr. Hanae Hosni
Research Team on Toxicology and Pharmacodynamics of Aromatic and Medicinal Plants ERTP. PAM, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V in Rabat, Morocco.

ABSTRACT

The aim of this work is to evaluate the acute toxicity and to study the anti-inflammatory effect of the leaves, fruits, and roots of *Asphodelus microcarpus.* Extraction by cold maceration with methanol was carried out on the different parts of the Am (leaves, fruits, and roots). The extracts obtained were subjected to an acute toxicity study to ensure their innocuousness. This study was carried out in accordance with the OECD guideline 423. Then an evaluation of the anti-inflammatory activity of these three methanolic extracts was carried out by two methods, a chemical method using carrageenan and another physical method by experimental trauma. The results showed that the methanolic extracts from the leaves, fruits, and roots of Am were found to be slightly toxic (with Letal dose for 50% determined at 5000 mg/kg), and they had a significant anti-inflammatory effect from the 200mg/kg dose for all three extracts. The low toxicity and possible anti-inflammatory power of the methanolic extracts from different parts of Am, imply today the need to isolate the active ingredients responsible for this activity in order to confirm the therapeutic interest of the said plant and subsequently propose possible galenic preparations.

KEYWORDS: *Asphodelus microcarpus*, anti-inflammatory, acute toxicity, carragenan, traumatism**.**

Résumé

L'objectif de ce travail est d'évaluer la toxicité aigüe et d'étudier l'effet anti inflammatoire des feuilles, des fleurs et des racines *d'Asphodelus microcarpus.*

Une extraction par macération froide au méthanol a été réalisée sur les différentes parties (feuilles, fleurs et racines) de l'Am. Les extraits obtenus ont fait l'objet d'une étude de la toxicité aigue afin de s'assurer de leurs innocuités. cette étude a été réalisé suivant la ligne directrice de l'OCDE 423.

Ensuite, une évaluation de l'activité anti-inflammatoire de ces trois extraits méthanolique a été réalisée par deux méthodes, une méthode chimique à la carragéenine et une autre méthode physique par traumatisme expérimental.

Les résultats ont prouvé que les extraits méthanoliques des feuilles, des graines et des racines de *l'Asphodelus microcarpus* se sont révélés faiblement toxiques (avec une Dose Létale à 50% égale à 5000mg/kg), et que les extraits méthanoliques de la plante médicinale *Asphodélus microcarpus* sont doués d'un effet anti-inflammatoire significatif à partir de la dose 200mg/kg pour les trois extraits.

La faible toxicité et l'éventuel pouvoir anti inflammatoire des extraits méthanoliques de différentes parties de l'*ASPHODELUS MICROCARPUS* impliquent aujourd'hui la nécessité d'isoler les principes actifs responsables de cette activité afin de confirmer l'intérêt thérapeutique de la dite plante et par la suite proposer d'éventuelles préparations galéniques.

mots clés: *Asphodelus microcarpus*, anti-inflammatoire, toxicité aigue, carragénéine, traumatisme.

INTRODUCTION

Morocco's privileged geographical situation with a double seafront and its orographic diversity with four major mountain ranges: the Rif, the Middle Atlas, the High Atlas and the Anti-Atlas with altitudes exceeding 2000, in the Rif, 3000 in the Middle Atlas and 4000 m in the High Atlas give it a great climatic variability accompanied by a great diversity of vegetation cover and a rich and varied flora.[1] This flora (vascular flora) includes 3913 species and 1298 subspecies (including 426 subspecies types), distributed among 155 families and 981 genera. The number of endemic species is 640 (16%) and 280 subspecies (32%).[2]

The Liliaceae family includes about 250 genera and 3 500 species[3], in this plant family, we find the genus Asphodelus which includes 18 species of which only 30% have been published for their traditional uses, namely A. aestivus, A. fistulosus, A. microcarpus, A. ramosus, and A. tenuifolius. and 50% have been the subject of phytochemical studies (A. acaulis, A. aestivus, A. albus, A. cerasifer, A. fistulosus, A. macrocarpus, A. microcarpus, A. ramosus, A. tenuifolius). However, there are no data on traditional uses of A. acaulis, A. albus and A. cerasiferus.[4]

These Asphodels are distributed from the Saharan bioclimate genera (Asphodelus refractus Boiss.) to the subhumid and wet high mountain bioclimate (Asphodelus ayardii Jahand. & Mayor).[5]

Inflammation or inflammatory reaction is the response of living, vascularized tissues to physical, chemical or biological aggression in order to maintain their integrity. It is a set of reaction phenomena occurring at the point of irritation by a pathogen. It usually results in four cardinal symptoms: redness, heat, pain, and functional damage as stated by Aulus Cornelius Celsius.[6]

The inflammatory reaction involves many biological systems that occur at varying times and degrees: biochemical reactions, cell activation, coagulation, fibrinolysis that aim to destroy or eliminate the foreign substance. However, too long or too important an activation can lead to more or less permanent alterations.[7],[8]

Inflammation is usually a beneficial process: its purpose is to mobilize the immune system to eliminate the pathogen and repair tissue damage. Sometimes inflammation can be harmful due to the aggressiveness of the pathogen, its persistence, the location of the inflammation, or abnormal regulation of the inflammatory process.

In traditional medicine, these Asphodels are used differently. Thus, swollen tuberous roots are used in ear instillations in the treatment of ear infections. The decoction of leaves in poultice is used against rheumatism.

To our knowledge, this is the first study to provide data that the morning methanolic extract evaluated for toxicity and inflammation. The objective of this work is to study the acute toxicity of Asphodelus microcarpus and to test its anti-inflammatory activity.

MATERIALS AND METHODS

Plant matériels

Plant sample of *Asphodelus microcarpus* was collected from Province Ain Aouda, Rabat city, in March 2015.

The plant material was identified by Dr. TALEB Mohammed Sghir and Dr. KHAMAR Halim, botanists at the Scientific Institute of Rabat and the specimen of this plant has been deposited in the Herbarium national of the Scientific Institute of Rabat(RAB) with n° RAB78996.

Sample preparation and extraction

A medicinal plant becomes useful only after having a certain transformation. The plants are used according to fresh or dry cases, stabilized or not. The purpose of the division is to reduce the bodies to more or less coarse fragments or more or less fine particles, or to a powder.

Methanol was chosen as an extraction solvent by maceration for two reasons: for its low vapor pressure and for its high rank in polarity, capable of producing the maximum of bioactive compounds.[9]

100g of each part of the plant (roots, leaves, and fruits) are macerated in the dark, in two liters of methanol 95% between 24h to 48h. The operation is repeated several times until total depletion of the plant material and depends on its richness in chlorophyll. The methanolic extracts are filtered on Whatman paper and then concentrated under reduced pressure at 60°C. using the evaporator. At this stage, the yield is calculated from the final extract in relation to the weight of the dry plant.

Percent of Yield was calculated as follows[10]:

$$\text{Extract yield \% 100} = (W1/W2)*100$$

Where, W1= Net weight of powder in grams after extraction and W2= Total weight of wood powder in grams taken for extraction.

ACUTE ORAL TOXICITY

Experimental animals

These are Swiss mice, adult females, aged from 2 to 2.5 months and weighing from 20 to 30g from the animal center of the Faculty of Medicine and Pharmacy of Rabat.

Injecting the solution

The test consists of a stepwise procedure with the use of three female mice per step. The method permits estimation of an LD50 with a confidence interval and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS)25. After a single dose administration, mice were placed in individual clear plastic cages and all animals were observed for possible mortality cases (24h) and behavioral changes followed by daily weight monitoring for 14 days.[11]

ANTI-INFLAMMATORY ACTIVITY

Experimental animals

Adult male Wistar strain rats weighing between 180 and 220 g are fasted 18 hours before the study of anti-inflammatory activity. These animals come from the animal centre of the Faculty of Medicine and Pharmacy of Rabat. The temperature of the animal centre is maintained at 20 ° C with a lighting cycle of 12 hours of light / 12 hours of darkness.[12],[13]

All animals had free access to water and standard diet. They were acclimatized at least one week before the experiments started.

The animals submitted to the oral administration of the extracts or drugs, fasted for 18h before the experiment (water was available). All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for the experimental procedure in 2010; CEE509.[14][15]

Experience

The evaluation of the anti-inflammatory activity of *Asphodelus microcarpus* was carried out by using on two edema models, namely edema by injection of 0.05 ml of 1% carrageenan into NaCl 9‰ under the plantar fascia and edema by experimental trauma by dropping a 50 g weight on the left rat paw.[16],[17]

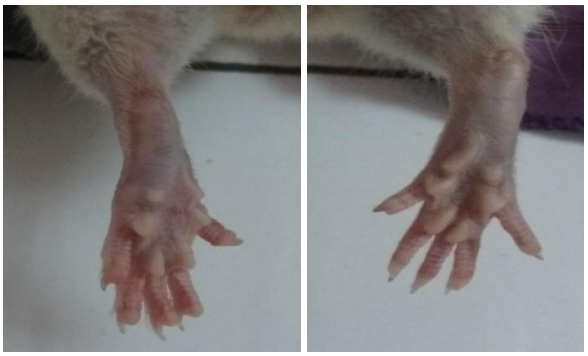
In both methods, all animals have fasted 18h before the test and received 5mL of distilled water by gavages to minimize individual variations in response to the swelling of the paws.

The right rat paw (RRP) is not treated, and it is taken as control.

- ✓ Carrageenan-Induced Rat Paw Edema.

The model of carrageenan-induced edema. The study rats received the various *Asphodelus microcarpus* extracts (100 and 200/kg) and indomethacin (10mg/kg) as a reference, distilled water (5mL/kg) was used as a negative control.

After 60 minutes, carrageenan was injected subcutaneously into subplate area of the animal's left rat paw (a). The right rat paw (b) is not treated, it is our witnesses. (figure 1).



a) LRP **b) RRT**

Figure 1: The LRP increased after oedema induced compared to the RRT that is control.

Then we noted the volumes of the inflammation at one hour 30 minutes, 3 hours and 6 hours.[18],[19] The percentages of inflammation inhibition have been calculated according to the following formula:

$$\%\ \text{of inhibition} = \frac{(\text{mean (Vleft} - \text{Vright)control} - (\text{Vleft} - \text{Vright)treated})}{(\text{Vleft} - \text{Vright)control}} \times 100$$

Where *V*left is the mean volume of edema on the left rat paw.

And *V*right is the mean volume of edema on the right rat paw.

✓ Experimental Trauma-Induced Rat Paw Edema.

Rats received the 200 mg/kg oral dose of the various methanolic extracts of *Asphodelus microcarpus*, the control group received 5 ml/kg of distilled water and the standard group received the reference drug (Indomethacin 10 mg/kg).

One hour later, a weight of 50 g was dropped on the animal's left rat paw. The right rat paw is not treated, it is taken as a control. then we note the volume of both legs, measured by a plethysmometer, at 1h30min, 3het 6h (14). The percentages of inflammation inhibition have been calculated according to the following formula:

$$\%\ \text{of inhibition} = \frac{(\text{mean (Vleft} - \text{Vright)control} - (\text{Vleft} - \text{Vright)treated})}{(\text{Vleft} - \text{Vright)control}} \times 100$$

(SE Mand analyzed by one-way analysis of variance (ANOVA) followed by Student's *t*-test. A value of $P < 0.001$ was considered significant).

3. RESULTS

Toxicité aigue

The initial doses of 300mg/kg of methanolic extract from the three parts of *Asphodelus microcarpus* (fruits, and roots) given orally with one repeat for each test did not induce any deaths. According to OECD 423 rules, the test doses were increased to 2000mg/kg (VO). With one confirmatory test for each extract and under the same experimental conditions, no deaths were also recorded.

Thus, based on the results obtained (Figure 2), and according to the OECD guideline for chemical testing - method by acute toxicity class, adopted on 17 December 2001, it can be concluded that the LD50 of méthanoliques extracts from the leaves, fruits, and roots of *Asphodelus microcarpus* is greater than 5000mg/kg VO.

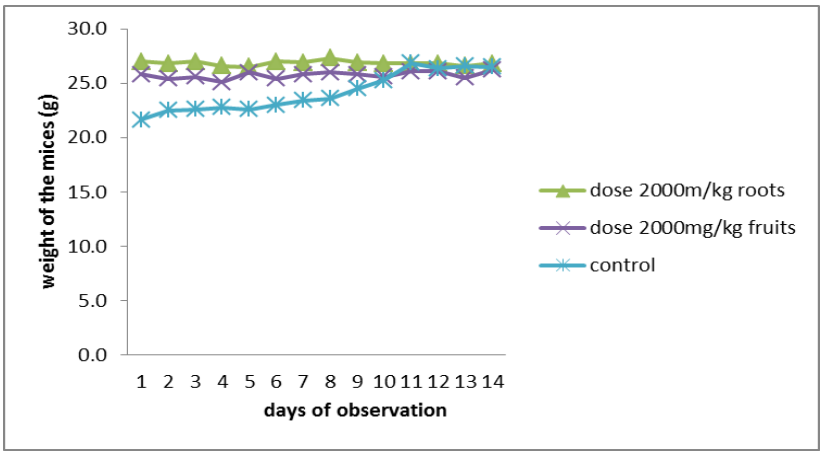


Figure 2: Evolution the mean weight of the mices.

1. Carragenan induced edema on the rat paw

Injection of carrageenan under the plantar fascia of the left rat paw of the control lot causes an increase in the average volume of the animal's paste by 0.17, 0.26 and 0.34 ml respectively at 1h 30min, 3h00 and 6h00 with a maximum at 6h00. Oral administration of indomethacin as a reference drug at a dose of 10 mg/kg significantly reduces the increase in the volume of the left rat paw with a volume of 0.04, 0.07 and 0.16 ml at 1h30min hours, 3h and 6 h after the carrageenan injection. Methanolic extracts from the three parts of the plant (roots, leaves, and fruits) are administered orally at a dose of 100 mg/kg, and at a dose of 200mg/ml only for

two parts of the plant (roots and leaves), induces a decrease in the volume of edema compared to the control at 1h 30min, 3h00 and 6h00 which suggests that the three parts of the plant have an anti-inflammatory action (Table 1).

Table 1: Effect of methanolic extract of roots, leaves and fruits of Asphodelus microcarpus on carrageenan induced rat paw edema.

Groups	Dose mg/kg v.o.	Mean volume of edema (left paw - right paw) carragenan-induced		
n=6		1h30	3h	6h
control		0.17 ± 0.01	0.26 ± 0.01	0.34 ± 0.02
Indomethacin	10	0.04 ± 0.004*	0.07 ± 0.005*	0.16 ± 0.008*
roots	100	0.13±0.02*	0.14± 0.03*	0.22 ± 0.05*
leaves	100	0.1±0.007*	0.19±0.02*	0.27±0.03*
fruits	100	0.16 ±0.02*	0.24 ± 0.03*	0.15±0.02*
roots	200	0.13±0.006*	0.06±0.01*	0.06±0.02*
leaves	200	0.04±0.01*	0.05±0.01*	0.07±0.01*

The evaluation of the inhibition percentage shows that extracts administered by VO at a dose of 100 mg/kg have a low anti-inflammatory activity compared to indomethacin, the reference drug.

The leaves extract has an inhibition at 41.18% in the initial phase of inflammation (1h30), then there is an exponential decrease in the anti-inflammatory action, unlike the roots extract which has a low inhibition after 1h30 and 6h i.e. in the initial and final phase, and a slightly high inhibition at 3h (acute phase) by 46.15%, while the seed extract has a very low percentage (5.88% and 7.69%) after 1h30 and 3h00 it has increased very significantly to 55.88% a value that is higher than that of indomethacin (52.94%) (Figure 3).

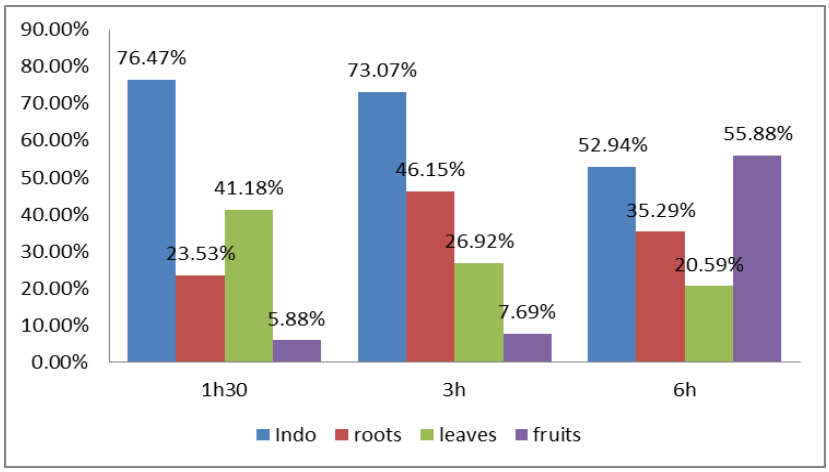


Figure 3: Percentage of inhibition of inflammation using carrageenan induced rat paw edema.

The evaluation of the inhibition percentage shows that the extracts administered by VO at the 200 mg/kg dose have a high anti-inflammatory activity compared to indomethacin is the reference drug.

Leaf extract has an inhibition 76.47% significantly similar to that of indomethacin 73.85% during the initial phase of inflammation, this inhibition has significantly increased and exceeded that of indomethacin at 3h and 6h by a percentage by 80.77% and 79.41%, while root extract has a low inhibition in the initial phase at 23.53%, but which becomes important during 3h and 6h (76.92% and 79.41%) and becomes higher than indomethacin (73.07% and 52.94%) (Figure 4).

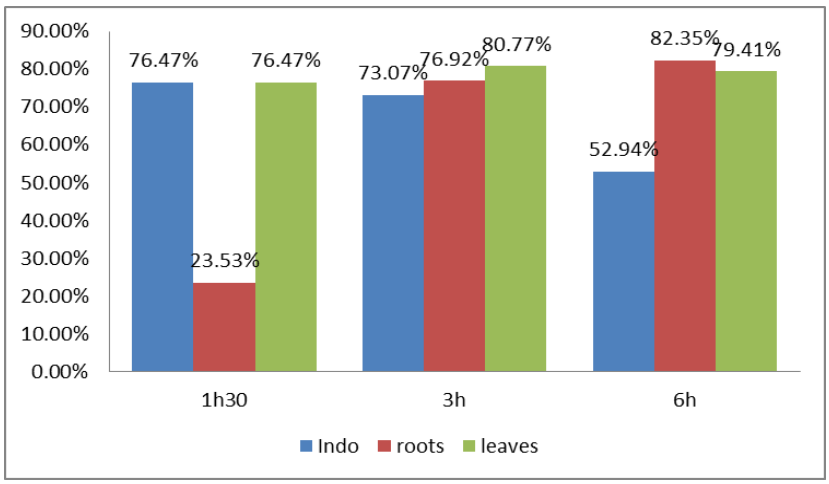


Figure 4: Percentage of inhibition of inflammation using trauma-induced rat paw edema.

2. Edema induced by experimental trauma

The traumatic shock on the left rat paw in the control batch results in a significant increase in the average volume of the edema by 0.153, 0.22 and 0.36 ml respectively at 1h30, 3h00 and 6h00. Indomethacin is the non-steroidal anti-inflammatory at a significantly reduced dose of 10 mg/kg ($p<0.05$) this increases in the volume of the edema by 0.04, 0.03 and 0.03 ml respectively at 1h30, 3h00 and 6h00. Methanolic extracts from the three parts of the plant (roots, leaves, and fruits) are administered orally at a dose of 200 mg/kg, induces a decrease in volume of edema compared to the witness at 1h30min, 3h00 and 6h00 which suggests that the three parts of the plant have an anti-inflammatory action (Table 2).

Table 2: Effect of methanolic extract of roots, leaves and fruits of Asphodelus microcarpus on trauma-induced rat paw edema.

Groups	Dose mg/kg v.o.	Mean volume of edema (left paw - right paw) trauma- induced		
n=6		1h30	3h	6h
control		0.15 ± 0.005	0.22 ± 0.02	0.36 ± 0.01
Indomethacin	10	0.04 ± 0.009*	0.03 ± 0.01*	0.03 ± 0.01*
roots	200	0.12 ± 0.02*	0.155 ± 0.03*	0.17 ± 0.01*
leaves	200	0.13 ± 0.02*	0.10 ± 0.02*	0.188±0.008*
fruits	200	0.09±0.01*	0.19 ± 0.06*	0.32 ± 0.06*

After the evaluation of the inhibition, percentage shows that the extracts administered by VO at a dose of 200 mg/kg have a low anti-inflammatory activity compared to indomethacin, the reference drug.

For root extract has significant inhibition at 52.78% at 6h00 , and the extract of the leaves its anti-inflammatory action is important at 3h at a percentage of 54.55%, while the seed extract has inhibition in the first ignition phase (1h30) to 40% then decreases exponentially after 3h and 6h (13.64% and 11.11%) (Figure5).

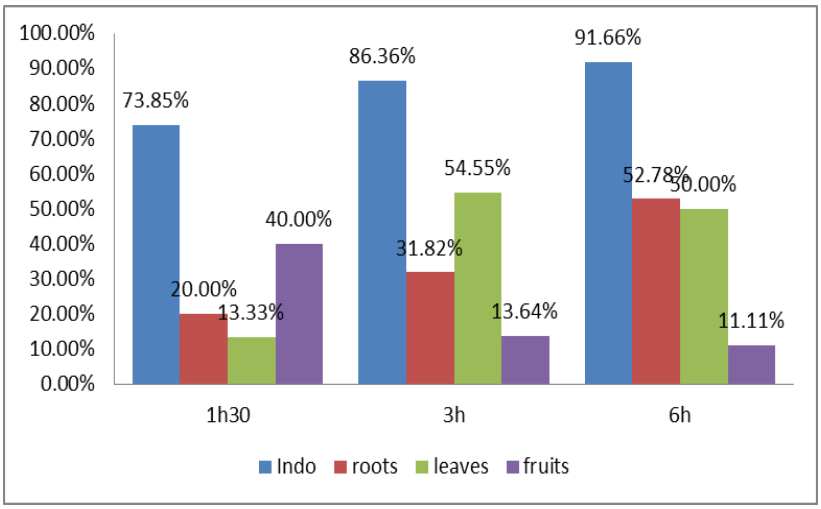


Figure 5: Percentage of inhibition of inflammation using trauma-induced rat paw edema.

DISCUSSION

According to the literature, Asphodel is a plant with pharmacological properties appreciable: diuretic, analgesic, anti-rheumatic and especially very effective in the treatment of ear infections, and based on research that has identified some of the most common molecules of its chemical composition (alkaloids, polyphenols, and flavonoids).

Since this plant is used in traditional Moroccan medicine and internally, a safety study was carried out by assessing the acute toxicity in vivo, which revealed that the LD50s of the methanolic extracts from the leaves[20], fruits, and roots of *Asphodelus microcarpus* are greater than 5000mg/kg VO, which gives the extracts a very low or even absent classified toxicity.

On the other hand, we have tried through our work to confirm "in vivo" its anti-inflammatory power.

Inflammation is a defensive reaction of the body. To this end, this study evaluated the anti-inflammatory properties of the methanolic extracts of *Asphodelus microcarpus* (roots, leaves, and fruits), we worked on two models: the carrageenan chemical edema induction model and the physical edema induction model by trauma. The analysis of the results shows the existence of an anti-inflammatory effect for all extracts performed but to varying degrees, extracts at the 200mg/kg dose in the carrageenan model showed similar or greater inhibition than indomethacin (reference drug) during the three inflammation phases, especially the late phase of inflammation.

The mechanism by which carrageenan induces the inflammatory process includes three phase: It stimulates the release of histamine and 5-hydroxytryptamine which promote vasodilation, plasma transudation, and edema; a second phase that uses kinins as mediators to increase vascular permeability and the phase whose mediator is supposed to be prostaglandin.[21] This release of prostaglandin is associated with leukocyte migration in the inflamed area. The prostaglandins are involved in inflammatory processes.

The extracts (roots, leaves, and fruits) showed different anti-inflammatory profiles depending on the model. This suggests that these extracts exercise a different modulation of the inflammatory mechanisms involved in each model.

Indeed, there are researchers who have worked on two models: carrageenin-induced sub-foot edema model in rats and ear edema model in rats induced by arachidonic acid in mice, and they showed that ethanol and chloroform from the roots of *Asphodelus microcarpus* showed significant activity on edema induced by arachidonic acid. However, they did not show any effect on carrageenan-induced edema.[22]

Carrageenan rat paw edema has been shown to be the most useful to date for studying inflammation involving prostaglandin mediation. Acid-induced edema in the ears of mice is generally insensitive to inhibition by cyclooxygenase inhibitors.[23]and[24]

Then it is suggested that *Asphodelus microcarpus* extracts have expressed this anti-inflammatory power through an inhibitory action that would be exerted more on cyclo-oxygenases that are responsible for prostaglandin synthesis. This inhibitory action may be due to flavonoids; researchers have indicated that flavonol is extremely effective in reducing edema.[25]

Therefore, further investigations are now required to establish the exact mechanism of action and identify the active ingredient(s) of each extract to explain their therapeutic efficacy.

Conclusion and prospects

Medicinal plants are still the reliable source of phytoactives known for their therapeutic properties, which leads us to the conservation of local plant biodiversity. And as phytotherapy is generating renewed interest, this work has focused on the study of the toxicity and anti-inflammatory effect of methanolic extracts from the leaves, fruits, and roots of the medicinal plant *Asphodelus microcarpus*.

The significant results obtained during this study showed that our plant has a low oral in vivo toxicity (LD50 > 5 000 mg/kg VO), which gives it the character of safety and also has an anti-inflammatory power at the 200mg/kg dose for the carrageenan edema induction model.

At the end of this information, we can only say, modestly, that we can confirm by our work a certain pharmacological property: "The anti-inflammatory effect" of *Asphodelus microcarpus*.

An earlier work by Razik & al(2016) found that the ethanolic extract of *Asphodelus microcarpus* 's roots also has anti-inflammatory properties.[26]

An experimental sequence is essential, which is based on the following axes:

- Make extractions differentiated by solvent gradient (polar, medium polar and apolar).
- Repeat the same tests for each family to target the active molecular margin.
- Study the action pathways of active molecules.
- Confirm the therapeutic interest.

- Propose possible galenic preparations.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Data Availability Statement

1. The botanical identification data of the plant used as well as a sample have been available at the National Herbarium of the Scientific Institute of Rabat (RAB) with No. RAB78996.
2. All the experimental data used to support the conclusions of this study are included in the article, for more details, do not hesitate to contact the author of this article.

ACKNOWLEDGEMENTS

The author Hanae HOSNI wishes to thank Pr Taleb Mohhamed Sghir and Dr Halim KHAMAR of scientific institute, Rabat for their help in identification of the species.

Also, we would like to thank Dr Kaoutar DIB for her valuable contribution and help specially for the English revision of this work.

Special thanks to all colleagues and professors at the Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco especially Dr. El Houcine Bouidida, Dr. Driss OURAINI and Pr. Zellou Amina their kind advice during the experimental work.

REFERENCES

1. La région circum-méditerranéenne. Centre mondial majeur de biodiversité végétale. Quezel, P., Medail F. s.l.: Gap. France, Actes 6èmes Rencontres de l'A.R.P.E., 1994; 152-161.
2. IbnTattou, M. , M. Fennane. Statistiques et commentaires sur l'inventaire actuel de la flore vasculaire du Maroc. Rabat: Université Mohammed V–Agdal, Institut Scient, 2012; 1-9, inventaire.
3. The Nature and Definition of an Alkaloid. Alkaloids: Chemical and Biological Perspectives, William Pelletier, S. Institute for Natural Products Research and The School of Chemical Sciences The University of Georgia, Georgia (USA), 1991; 7: 26.
4. Review: A Comprehensive Review on the Medicinal Plants from the Genus Asphodelu. Malmir, M., R. Serrano, M. Caniça & al., PLANTS, 2018; 7: 20.

5. flore vasculaire du Maroc inventaire et chorologie Volume 2 asteraceae et monocotyledones . Fennane, Ibn Tattou M & M. 2008, Travaux de l'Institut Scientifique, Série Botanique n°, 39.
6. Physiopathologie de la fièvre, de la douleur et de l'inflammation; Analgésiques, antipyrétiques, anti-inflammatoires et immunosuppresseurs (in Pharmacologie des concepts fondamentaux aux applications thérapeutiques). Schorderet, M , J-M. Dayer. & al., slatkine, 1998; 569-606.
7. Lechat P., F.Calvo, P. De Crémoux, J-P.Giroud, G.Lagier, Ph.Lechat et B.Rouveix, S. Weber. Les médicaments de l'inflammation, in Abrégé de pharmacologie. Paris: Masson, 1990; 288-319.
8. Dieng, C. Contribution à l'étude de Khaya senegalensis (DESR.) A.JUSS (Meliaceae). . Dakar: s.n., 1993; 09. P., thèse de pharmacie.
9. Comparison of extracts prepared from plant by-products using different solvents and extraction time. Brigita, L. , P. Mirko, W. Alenka Golc,. Journal of Food Engineeri, 2005; 214–222.
10. Phytochemical evaluation and bactericidal potential of Terminalia arjuna stem bark. Patil, U.H, D.K. Gaikwad., International Journal of Pharmaceutical Sciences and Research, 2010; 614-619.
11. Toxicité orale aiguë: Méthode par classe de toxicité aiguë. OCDE, Ligne directrice de L'OCDE pour les essais de produits chimiques, 2001.
12. Manuel sur le soin et l'utilisation des animaux d'expérimentation. Olfert, ED. BM. Cross, AA. Mc William. 1993. Conseil canadien de protection des animaux.
13. Laroche, MJ, Rousselet F. Les animaux du laboratoire: éthique et bonnes pratiques. Paris: Masson, 1990.
14. In Vivo Potential Anti-Inflammatory Activity of Melissa officinalis L. Essential Oil. Bounihi, A, Hajjaj,G, Alnamer, R, Cherrah, Y, Zellou,A., Hindawi Publishing Corporation Advances in Pharmacological Sciences, 2013; 7.
15. Psychostimulant activity of Rosmarinus officinalis essential oils. Alnamer, R., K. Alaoui, H. El Bouidida, A. Benjouad, and Y.Cherrah,. Journal of Natural Products, 2012; 83–92.
16. Activité analgésique et anti-inflammatoire des saponines d'Argania spinosa. Alaoui, K., JF. Lagorge, Y. Cherrah, et al., Ann Pharm Fr, 1998; 220–8.
17. Antiinflammatory activities of methanolic extract of Jatropha unicostata balf (Sibru) and ethanolic extract of Aloe perryi baker (taife), as endemic plants in Yemen. Al-sobarry, M., A. Alwashli A, Cherrah Y, Alaoui K., Int Nat Prod Sci., 2011; 40-55.

18. Analgesic and anti inflammatory activities of Boswellia elongata balf methanolic extracts, as endemic plants in Yemen. Alwashli, A., Al-sobarry M. , Alnamer R., Cherrah Y., and Alaoui K., Journal of Biologically Active Products from Nature,, 2012; 90-98.
19. Anti-inflammatory effect of lycopene on carrageenan-induced paw oedema and hepatic ischaemia-reperfusion in the rat. Bignotto, L., Rocha, J., Sepodes B., British Journal of Nutrition, 2009; 126–133.
20. Toxicité aigue, cytotoxicité et effet anti-radicalaire de l'extrait méthanolique des feuilles de l'asphodèle, Asphodelus microcarpus. HOSNI, H., SALAMA, A., ABUDUNIA, A. CHERRAH, Y., IBRAHIMI A., ALAOUI K. 2018, Phytothérapie (under review).
21. Preventive effects of Unsei-in and Oren-gedoku-to, Chinese traditional medicines, against rat paw oedeme and abdominal contriction in mice. Wang, LM, S. Mineshita., J Pharm Pharmacol, 1996; 327-331.
22. Anti-inflammatory Activity of Some Extracts from Plants used in the Traditional Medicine of NorthAfrican Countries. Rimbau, V., Risco,E. Canigueral S.and Iglesias J, phytotherapy research, 1996; 421-423.
23. Arachidonic acid-induced inflammation: inhibition by dual inhibitor of arachidonic metabolism. Griswold., D.E, E. Webb, L. Schwartz and N. Hanna,. 190-199, Inflammation, 1987.
24. Correlation between mouse skin inflammation induced by arachidonic acid and eicosanoid synthesis. Chang, J., Carlson, R. P., O'Neill-Davis, L., Lamb, B., Sharma, R. N., & Lewis, A. J., lnflammation, 1986; 205-214.
25. Anti-inflammatory properties of plant flavonoids Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Guardia, T., Rotelli, A. E., Juarez, A. O., & Pelzer, L. E., Il farmaco, 2001; 683-687.
26. Antioxidant anti-inflammatory and antibacterial activities of Asphodelus microcarpus,. Razik, A., Adly, F., Lahlou F.Z, Hmimid. F, Fahde, S., world journal of pharmaceutical research, 2016; 666-673.