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Identification of the Anti-inflammatory and Wound Healing Effects of *Cordyceps militaris* Extract in Animal Models

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Abstract *Cordyceps militaris* is one of the most well known medicinal entomopathogenic fungi and as such has been widely used for the treatment of various diseases. However, the pharmacological and biochemical activities of *C. militaris* have not been clearly elucidated. The evaluation of the anti-inflammatory activity of *C. militaris* was performed by the test for plantar edema induced by injection of carrageenan in mice. The percentage inhibition of edema and the percentage increase in paw edema volume were then evaluated. Wound healing effect of *C. militaris* extract was studied on an excision wound model in rats. Changes in wound area were measured regularly and the rate of wound contraction calculated, then the histopathological recovery was evaluated. The results in all animals were not shown signs of toxic effects, moribund, and mortality. Oral administration of *C. militaris* extract reduced significantly after 2 hours the paw edema induced by carrageenan in mice (8.96±2.83%, 6.25±2.70% and 4.92±2.22 % at doses of 100, 200 and 300mg/kg BW respectively); the histological study confirmed the decrease in inflammatory response. Topical application of *C. militaris* ointments at 5% and 10% showed an enhance rate of contraction and significantly decreased wound area than control group. With histological evidence of more collagen formation in the skin and early epithelization period with low scar area. *C. militaris* mushrooms definitely deserve to be considered as functional foods and also have great potential for medicinal use.

Keywords: *Cordyceps militaris, anti-inflammatory, wound healing, mice, rat*

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1. Introduction

Therapeutic mushrooms continue to be a significant part of human culture and have intriguing health benefits [1]. With about 750 species, the genus Cordyceps is one of the largest genera in the Clavicipitaceae family. It is also one of the most diversified in terms of variation in species, morphology, and ability to adapt to a variety of hosts [2]. The medicinal mushroom *Cordyceps militaris* found in East Asia, has been widely consumed in China for medication purposes since ancient times (3000 years) [3,4]. Due to its rarity, many investigators have been devoted to focused on the artificial cultivation of *C. militaris* using cutting-edge technology [5].

The mycelium and fruiting bodies of *C. militaris* are ideal food supplements and pharmaceutical agents and conserve as a good source of prohealth substances potentially bioavailable for humans. Studies on the fruiting bodies and mycelia of *C. militaris* have shown the presence of biologically active substances, such as γ-aminobutyric acid, ergothioneine, sterols (ergosterol), statins (lovastatin), phenolic compounds (including phenolic acids and flavonoids), vitamins, and bioelements [6]. Cordycepin, peptides, polysaccharides, and other active compounds produced from *C. militaris* will be a key factor in the development of green pharmacognosy and pharmacology [7]. These numerous bioactive compounds obtained from this fungus have been looked as responsible for their biological and therapeutic properties, with their antitumor, and immunomodulatory effects [8,9,10,11,12], anti-inflammatory activity [13], and antioxidant activity [14].

Inflammation plays a crucial role in biological processes that are triggered by a variety of stimuli and harmful elements, including UV radiation, irritants, infections, and cell damage. Redness, a rise in body temperature, pain, and changes in physiological processes at infected locations are the key characteristics of inflammation [15,16]. Antioxidant function, transcription factors, matrix metalloproteinases, complement cascade characteristics, and adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), selectin, and vascular cell adhesion molecule-1 (VCAM-1) are all components of inflammatory responses. Additionally, *C. militaris* and its contents control the expression of inflammatory genes as well as the actions of

proinflammatory enzymes.

Cordyceps species, its extracts, and bioactive constituents have been related with cytokine production such as interleukin (IL)-1β, IL2, IL-6, IL-8, IL-10, IL-12, and tumor necrosis factor (TNF)-α, phagocytosis stimulation of immune cells, nitric oxide production by increasing inducible nitric oxide synthase activity, and stimulation of inflammatory response via mitogen-activated protein kinase pathway [17]. Pre-clinical research has revealed several pharmacological effects, including antioxidant, anti-cancer, antihyperlipidemic, antidiabetic, anti-fatigue, anti-aging, hypocholesterolemic, hypotensive, vasorelaxant, anti-depressant, aphrodisiac, and kidney protection. The bioactive substances found in Cordyceps, such as nucleosides, sterols, flavonoids, cyclic peptides, phenolics, bioxanthracenes, polyketides, and alkaloids, are associated with these biological activities [18]. The research involving *C. militaris*-derived polysaccharides has increased rapidly, particularly in their structural characterization and pharmaceutical activities including antioxidant, immunomodulatory, and antitumor activities [19].

In recent years, naturals are being employed in wound treatment because of their ability to promote blood clotting, fight infection, and accelerate wound healing. These bioactive agents usually modulate one or more phases during healing processus [20]. Extracts from medicinal mushrooms and their metabolites have been verified for wound treating with contribution to different mechanisms of the healing process [21]. Wounds are physical injuries that result in an opening or breaking of the skin. Skin wound healing shows an extraordinary cellular function mechanism, unique in nature and involving the interaction of several cells, growth factors, and cytokines [22]. The wound healing of mushrooms has been also increasingly reported, linked to their richness in polysaccharides and phenolic compounds [23].

Therefore, substantial attention has recently been given to the development of natural compounds for being employed in anti-inflammatory and wound treatments with greater safety and minimal side effects. In the current study, research activities related to anti-inflammatory and wound healing exhibited by *C.militaris* extract was conducted in animal models.

2. Materials and Methods

2.1. Fungal Material

Aqueous extract of the medicinal Mushroom *Cordyceps militaris* (L.) (Sk gold) was purchased from the Institute of natural products of Hanoi, Vietnam Academy of science and technology. The determination of the concentrations of mineral elements in *C. militaris* extract was measured by a graphite furnace atomic absorption spectroscopy (AA-7000, SHIMADZU).

2.2. Animal Material

Female mice NMRI (25-30g) and male Wistar rat (250-270g) were obtained from the Pasteur institute (Algiers, Algeria). Animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature at 25±2°C with 12:12 light: dark cycles) and water ad libitum. Food was provided by dry pellets. This study was approved in accordance with the Algerian Legislation (Law Number 17-457/2019) inherent to the protection of animals designed to experimental and other scientific purposes as well as with the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA).

2.3. Acute Oral Toxicity

Acute toxicity study of C. militaris was assessed in both mice and rats by using an acute oral toxic class method of Organization of Economic Co-operation and Development (OECD), guidelines (OECD/OCDE, 2000). Briefly, the C. militaris powder were calculated at gradual doses (300 mg, 500 mg, 1000mg/kg body weight) for mice and a unique dose (5000 mg/kg body weight) for rats and freshly mixed with distilled water as a vehicle prior to administration. All animals were fasted 18 h before testing and divided into two groups, the control group received only the vehicle, and the test group received C. militaris orally by gavage using a stainless steel stomach tube. Animals were then observed for any manifestation of toxicity, increase in locomotor activity, salivation, convulsions, coma, and death at first 15, 30, and 60 minutes then daily for 14 days.

2.4. *In vivo* Anti-inflammatory Activity

Anti-inflammatory properties of *C. militaris* were performed according to the recommended methods of Alwashli et al. [24] and Bignotto et al. [25]. A total of thirty healthy female mice NMRI were fasted 18 h before testing and divided into five groups of six mice each. The duration of experimentation was 6 hours, with each group receiving the experimental solutions orally as follows: In group 1 - control group (Physiological water, NaCl 0,9%), group 2 - (Diclofenac dissolved in NaCl 0,9% with concentration of 10ml/kg), group 3 - Low dose of aqueous extract of *C. militaris* (100mg/Kg body weight of *C. militaris* dissolved in NaCl 0,9%), group 4 – middle and group 5 - high dose (200mg/Kg and 300mg/Kg body weight of *C. militaris* dissolved in NaCl 0,9%, respectively). The dosage of administration to each animal was calculated based on the body weight of the animal prior to administrate at a constant volume not exceed 1 ml per 100 g body weight. One hour after the administration of the treatments, each animal in all groups received, by sub-plantar injection in the right hind paw, 0.1 ml of a 0.1% carrageenan a highmolecular-weight polysaccharide induced paw edema model dissolved in 0.9% NaCl.

The percentage increase in paw edema volume (% AUG) for each group of mice was calculated by measuring the diameter of the paw, using a digital micrometer before and after induction of inflammation at intervals of one hour to six hours, it is given in the equation (1) below. Then the percentage inhibition of edema (% INH) was calculated for each group of mice treated relative to the control as given in the equation (2).

$$\%AUG = \left[(Dn - Do) \times 100\right] / Do \quad (1)$$

Dn: Diameter of the leg after the injection of carrageenan and DO: Diameter of the leg before the injection of carrageenan.

$$\%INH = \left[(\%AUG\ control - \%AUG\ treated) \times 100\right] / \%AUG \quad (2)$$

2.5. *In vivo* Wound Healing Activity

The preparation of the ointments to be applied was carried out by drawing up first the cream, wich was prepared by mixing an aqueous phase (xanthan gum in distilled water) with a fatty phase (sunflower oil, emulsifying wax, and conservative). The base cream was then added to the medicinal mushroom extract under sterile conditions to create the *C. militaris* ointments with 5 and 10% densities. The *C. militaris* ointments were topically applied to assess any manifestation of inflammation (erythema and edema).

Rats were anesthetized with an intraperitoneal (i.p.) injection of ketamine (50 mg/kg) and diazepam (1 mg/kg), and after the back was shaved, an area of about 2 cm^2 was marked by a standard ring above the spin. Full-thickness of the marked skin was then cut carefully. 24 hours after creation of open excision wounds, the animals were divided into five groups of five rats each. The rats were subjected to daily application of the experimental preparations in to the wound at the same time once a day for 21 days as follows: In group 1 - control group (non treated rats), group 2 – placebo (rats treated with 0,5 g of base cream), group 3 – standard (rats treated with 0,5 g of madecassol), group 4 – low dose (rats treated with 0,5 g of *C. militaris* ointment 5%), group 5 - high dose (rats treated with 0,5 g of *C. militaris* ointment 10%).

The evolution of the lesions was evaluated using the healing assessment parameters which are epithelization, exudate, erythema and scar. The wounds were observed and photographed every 3 days at the same time and under the same conditions until their complete closure. To determine the wound area, a transparent acetate paper was placed directly on the wound, and its outline was traced with a fine-tipped marker and the number of squares in the outline were counted [26]. Changes in wound area were monitored planimetrically by tracing the wound margin on graph paper regularly and the rate of wound contraction was calculated as given in the equation (3).

$$\%\ of\ wound\ inhibition = \left[\frac{Healed\ area^*}{Total\ wound\ area}\right] x100 \quad (3)$$

*Healed area = original wound area − present wound area.

2.6. Histological Study

Six hours after the induction of inflammation in mice and after the 21st day for histopathological examination of skin in rats, the animals were euthanized using CO_2 chamber and the animals death was confirmed by assessing the heart rate and respiration. The process of euthanasia was conducted following the AVMA guidelines for the euthanasia of animals: 2020 edition. Tissue samples were taken, fixed in 10% buffered formalin, processed, decalcified with 2% nitric acid solution for three hours, blocked with paraffin, then sectioned into 5 µm sections, and stained with hematoxylin and eosin.

2.7. Statistical Analysis

Statistical comparison was performed using one-way analysis of variance (ANOVA) followed by the paired Student's t-test in the XLSTAT software, and difference was considered significant for $P<0.05$.

3. Results

3.1. Anti-inflammatory Activity

The evaluation of the anti-inflammatory activity of *C. militaris* was performed by the test for plantar edema induced by injection of carrageenan in mice. The percentage increase in paw edema volume (%AUG) that carrageenan administration significantly increased was calculated for all treated groups of mice (Figure 1). Oral administration of the aqueous extract of *C. militaris* at doses of 100, 200, and 300mg/kg BW seems to significantly reduce carrageenan-induced edema at the second hour (8.96±2.83%, 6.25±2.70% and 4.92±2.22 % respectively). At the third hour, this decrease is greater in mice treated with high doses (3.13±1.80% at 200mg/kg BW and 1.27±0.9% at 300mg/kg BW), values which remained significantly reduced compared to the values of the second, third and fourth hour in the group treated at the highest dose of 300mg/kg BW ($P < 0.01$).

The results of the percentage inhibition of edema (% INH) indicate that after the first and second hour (Figure 2), the treatment with the oral administration of the *C. militaris* extract at the doses 200 and 300 mg/kg BW caused a significant inhibitory activity in carrageenan induced paw inflammation compared to the group treated with the extract at a dose of 100 mg/kg BW and standard group ($P <0.01$).

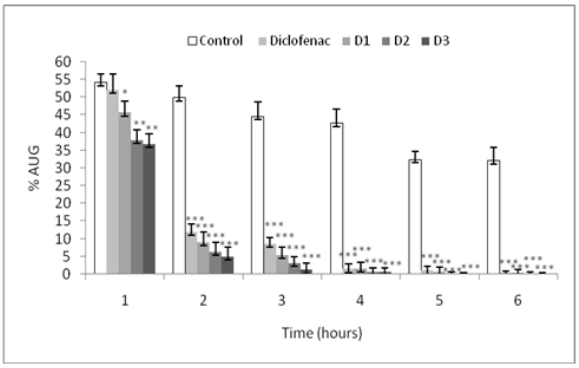

Figure 1. The percentage increase in paw edema volume (%AUG). Mice were divided randomly into five groups: Control Carrageenan group, Diclofenac group (50mg/kg), *C. militaris* (D1) groups (100mg/kg), *C. militaris* (D2) groups (200mg/kg) and *C. militaris* (D3) groups (300mg/kg). Results are expressed as the mean±SD (n=6 animals per group). *p<0.05, **p<0.01, ***p<0.001 were considered significant when compared with the Control carragenan group.

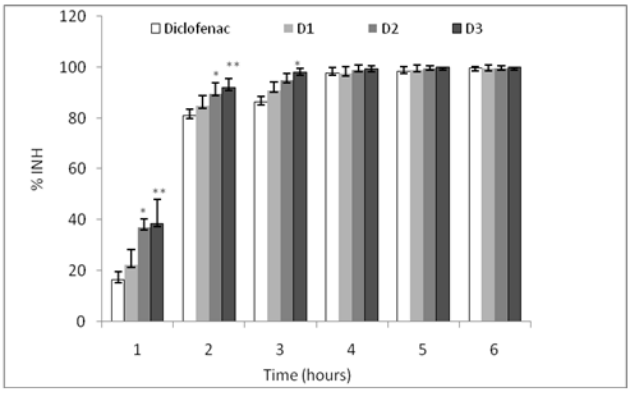

Figure 2. Effect of *C. militaris* extract on percentage inhibition of edema (% INH). Mice were divided randomly into five groups : Control carrageenan group, Diclofenac group (Mice were divided randomly into five groups: Control Carrageenan group, Diclofenac group (50mg/kg), *C. militaris* (D1) groups(100mg/kg), *C. militaris* (D2) groups (200mg/kg) and *C. militaris* (D3) groups (300mg/kg). Results are expressed as the mean±SD (n=6 animals per group). *p<0.05, **p<0.01, ***p<0.001 were considered significant when compared with the standard group (Diclofenac)

3.2. Wound Healing activity

Irritation test of *C. militaris* extract observed after application on the backs of rats indicated no signs of inflammation (Erythema or edema). The primary cutaneous irritation index obtained allows this extract to be considered as non-irritating (data not shown).

The Wound contraction assessed by comparison of the score's means of macroscopic appearance of the wounds from day 0 to day 21 revealed an improvement in the appearance of the wounds with a progressive reduction in erythema and exudate observed in all groups (Table 1). Regarding erythema, a significant appearance was shown from day 12 ($P<0.05$) and day 3 ($P<0.001$) for the placebo and Madecassol groups, respectively, which then tended to decrease beyond day 15 until day 21. Topical application of *C. militaris* ointment (5%) showed a significant appearance of erythema compared to the control group from day 6, significance was observed ($P<0.01$) until day 21. For the group of *C. militaris* (10%), this appearance became significant ($P<0.01$) from day 9 to day 21. The exudate present in the control and placebo groups in the first 3 days was absent in each of the Madecassol and *C. militaris* (5% and 10%) groups. The appearance of buds began on day 3 in all groups and increased in each of the control and placebo groups until day 9, for the Madecassol and *C. militaris* (5% and 10%) groups the increase noticed until day 12 was higher than that of the control and placebo. The epithelization phase began on day 3 in all groups, it increased in each of the control and placebo until day 9, then gradually decreased in the following days. This increase was higher in the Madecassol and *C. militaris* groups (5% and 10%), from day 12 a rapid gradual decrease was noticed until the last day of observation. Scab formation started from day 3 in all treated rats, then increased in each of the control and placebo lots until day 9. In the treated groups of madecassol and *C. militaris* (5% and 10%) a higher increase was noticed ($P<0.01$) until day 12, followed by a more rapid gradual decrease observed until the last day of the test.

Table 1. Effect of topical aplication of ointments on erythema, exudate, appearance of buds, epithelization period and scar area in excision wound. *p<0.05, **p<0.01, ***p<0.001 were considered significant when compared with the Control group.

	Ointment application	0 day	3th day	6th day	9th day	12th day	15th day	18th day	21th day
Erythema	Control	3.6 ±0.5	3 ± 0	3± 0	3 ± 0	2.8 ± 0.4	2.6 ± 0.5	2.4 ± 0.5	2.2 ± 0.4
	Placebo	3.8 ± 0.4	2.8 ± 0.4	2.4 ± 0.5	2 ± 0.7	1.8 ± 0.4*	1.4 ± 0.5*	1.4 ± 0.5*	1.2 ± 0.4*
	Madecassol	3 ± 0	2 ± 0***	1.6 ± 0.5**	1.2 ± 0.4***	1.2 ± 0.4***	1.2 ± 0.4**	1.2 ± 0.4**	1 ± 0**
	C. militaris 5%	3.8 ± 0.4	2.8 ± 0.4	2.2 ± 0.4**	1.8 ± 0.4**	1.4 ± 0.5**	1.2 ± 0.4**	1 ± 0**	0.6 ± 0.5**
	C. militaris 10%	3 ± 0	2.2 ± 0.4**	2 ± 0.7	1.4 ± 0.5**	1.2 ± 0.4**	1.2 ± 0.4*	1 ± 0**	0.8 ± 0.4**
Exudate	Control	0.8 ± 0.4	0.8 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Placebo	0.2 ± 0.4	0.2 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Madecassol	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C. militaris 5%	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C. militaris 10%	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Appearance of buds	Control	0 ± 0	1 ± 0	1.4 ± 0.5	2 ± 0	1 ± 0	1 ± 0	1.6 ± 0.5	1 ± 0
	Placebo	0 ± 0	1 ± 0	1.6 ± 0.5	2 ± 0	1.8 ± 0.4***	1 ± 0	0.8 ± 0.4	0.8 ± 0.4
	Madecassol	0 ± 0	1 ± 0	2 ± 0	2.4 ± 0.5	3 ± 0***	3 ± 0***	1.6 ± 0.5	0.4 ± 0.5*
	C. militaris 5%	0 ± 0	1 ± 0	1.4 ± 0.5	2.4 ± 0.5	3 ± 0***	2.6 ± 0.5**	1.6 ± 0.5	0.6 ± 0.5
	C. militaris 10%	0 ± 0	1 ± 0	2 ± 0	2.6 ± 0.5	3.4± 0.5 ***	3 ± 0***	2 ± 0	0.2 ± 0.4*
Epithelization period	Control	0 ± 0	1 ± 0	1 ± 0	1.2 ± 0.4	1.8 ± 0.4	1.6 ± 0.5	1 ± 0	1 ± 0
	Placebo	0 ± 0	1 ± 0	1.6 ± 0.5	1.6 ± 0.5	2 ± 0	1.2 ± 0.4	0.8 ± 0.4	0.8 ± 0.4
	Madecassol	0 ± 0	1 ± 0	2 ± 0***	2.4 ± 0.5*	3 ± 0*	2.4 ± 0.5	1.6 ± 0.5	0.4 ±0.5*
	C. militaris 5%	0 ± 0	1 ± 0	1.4 ± 0.5	2.4 ± 0.5*	3 ± 0***	2.4 ± 0.5	1.4 ± 0.5	0.4 ± 0.5*
	C. militaris 10%	0 ± 0	1 ± 0	2 ± 0 ***	2.6 ± 0.5*	3.4 ± 0.5**	2.4 ± 0.5	1.2 ± 0.4	0.2 ± 0.4*
Scar area	Control	0 ± 0	0.4 ± 0.5	1 ± 0	1.2 ± 0.4	1.6 ± 0.5	1.4 ± 0.5	1 ± 0	1 ± 0
	Placebo	0 ± 0	1 ± 0	1.6 ± 0.5	2.2 ± 0.8*	1.6 ± 0.5	1.2 ± 0.4	0.8 ± 0.4	0.6 ± 0.5
	Madecassol	0 ± 0	1.4 ± 0.5	2.4 ± 0.^{5**}	3.4 ± 0.5**	2.4 ± 0.5	1.4 ± 0.5	0.4 ± 0.5	0 ± 0***
	C. militaris 5%	0 ± 0	1.6 ± 0.5	2.8 ± 0.4***	3.4 ± 0.5**	2.4 ± 0.5	2.2 ± 0.4	1.2 ± 0.4	0.2 ± 0.4*
	C. militaris 10%	0 ± 0	1.6 ± 0.5	2.6 ± 0.5**	3.6 ± 0.^{5***}	2.8 ± 0.4*	1.8 ± 0.4	0.8 ± 0.4	0 ± 0***

Macroscopic aspect of wounds appeared less inflamed with the evolution of the days, those treated with *C. militaris* showed the best evolution compared to the other treatments (Figure 3). Rates of wound contraction observed at day 21 were 78,14%, 82,07%, 97,28%, 97,86%, and 98,68% in control rats, placebo, Madecassol, *C. militaris* 5% and *C. militaris* 10% respectively (Figure 4). The wounds treated with Madecassol and *C. militaris* (5%) ointments showed a significant surface area compared to the control in the first 3 days ($P<0.05$), from day 6, a significant increase in surface compared to the control ($P<0.001$). From day 9 to day 12 a significant increase in surface area was shown ($P<0.01$), after day 15 until day 21 a highly significant increase ($P<0.001$) was observed. Wounds treated with *C. militaris* ointment (10%) revealed a highly significant increase in surface area within 21 days compared to the control ($P<0.001$).

Histology of excision biopsy of skin wound showed healed skin structures in both madecassol and C. militaris (5% and 10%) treated groups, in which were observed after day 21 of the topical application of the ointment treatment, the dermis and epidermis with formation of the amount of ground substance in the granulation tissue (Figure 5).

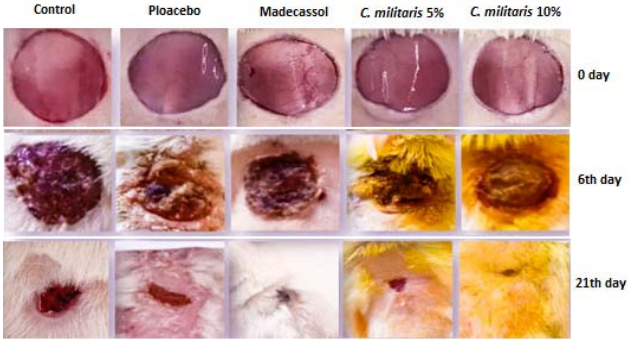

Figure 3. Photographic representation of contraction rate showing percent wound contraction area on different post excision days of control, placebo, madecassol, *C. militaris* 5% and 10% treated rats.

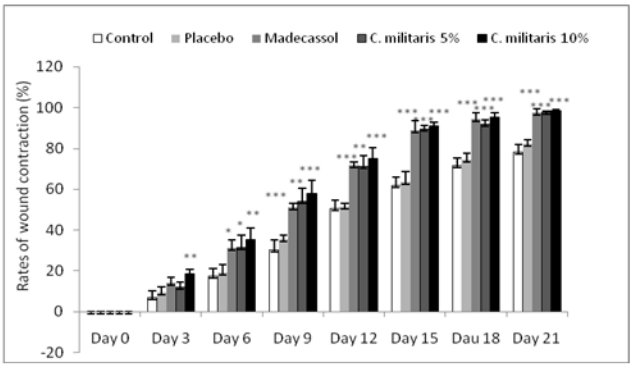

Figure 4. Rates of wound contraction. Results are expressed as the mean±SD (n=5 animals per group). *p<0.05, **p<0.01, ***p<0.001 were considered significant when compared with the Control group.

4. Discussion

The detection of mineral elements using atomic absorption method showed that the content of manganese (Mg), cadmium (Cd), copper (Cu), and zinc (Zn) in *C. militaris* extract, respectively, is 2.0227 µg/g, 17.9078 µg/g, 0.0187 µg/g and 0.1346 µg/g. The content of heavy metals in cultivated fruiting bodies of *C. militaris* is within the limits of Chinese national food safety standard [27]. Moreover, trace elements are essential to immune regulation and infection prevention [28].

The acute oral toxicity of *C. militaris* in rats (5000 mg/kg BW) and mice (300 mg, 500 mg, 1000mg/kg BW) was not shown signs of toxic, moribund and mortality in all animals. Thus, the health observation, feed and drinking water consumptions, was normal. These findings are consistent with the study by Long et al. [29], who worked on the toxicity of Chinese Cordyceps. *C. militaris* was recently classified in GHS category 5 or unclassified, the LD50 cut off at 5000 mg/kg body weight to infinity (∞) [30]. In our study, the analyzed parameters of the hematological analysis and clinical biochemistry analysis were not shown toxic to *C. militaris* in Wistar rats (data not shown).

In the first part of this study, the anti-inflammatory activity of *C. militaris* was evaluated in mice at dose level 100, 200, and 300 mg/kg BW. Results obtained showed that the aqueous extracts of *C. militaris* appreciably reduce the induced paw edema by carrageenan 1%, this inhibition is comparable to that of Diclofenac. Carrageenan is a mucopolysaccharide that induces local inflammation following its injection, the cause of this reaction is tissue damage which induces the synthesis of histamine, prostaglandins, leukotrienes, PAF (platelet activating factor), cytokines, NO (monoxide nitrogen) and TNF [31]. In addition, many studies seem to indicate that LPS is a potent macrophage activator related to the release of a variety of cytokines produced by cells and trigger or enhance the specific inflammation response [32].

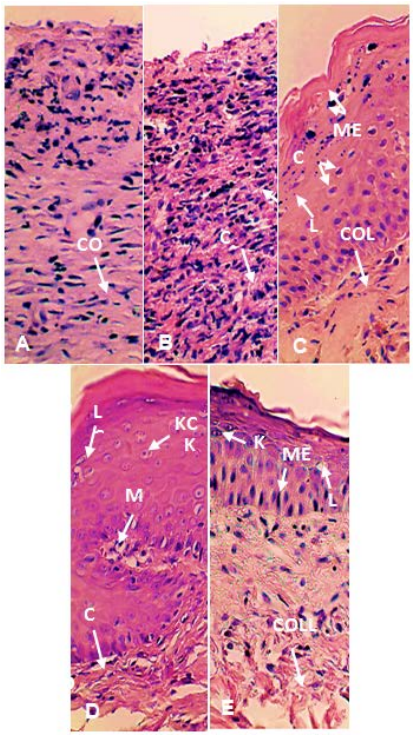

Figure 5. Histopathology of skin ×40. A: Control rat. B: Base cream treated rat (Placebo). C: Madecassol treated rar. D : *C. militaris* (5%) treated rats. E: *C. militaris* (10%) treated rats. LC: Langerhas Cells; KC: Keratinocytes; MEL: Melanocytes; COLL: Collagene.

Microscopic observation of the treated mice with the aqueous extract of *C. militaris* at the lowest dose (100mg/kg BW) and the highest dose (200mg/kg BW) showed a skin covering within the normal histological limit with almost total disappearance of the inflammatory infiltrate, at the highest dose (300mg/kg BW) the disappearance of edema and tissue congestion with a decrease in the intensity of the inflammatory infiltrate were observed (data not shown). The richness of the aqueous extract of *C. militaris* in different chemical constituents can justify this anti-inflammatory activity [33]. The actual mechanisms involved in anti-inflammatory responses include antioxidant activity, transcription factors, matrix metalloproteinases, complement cascade properties, and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), selectin, and vascular cell adhesion molecule-1 (VCAM-1) [7]. In subsequent *in vitro* tests, the anti-inflammatory activity of *C. militaris* was confirmed to result from the inhibition of the production of proinflammatory mediators (NO,TNF-α and IL-6), which were induced by LPS in murine macrophages, the group of bioactive compounds of C. *militaris* was not defined in the study [34]. Recent studies showed that COX-2 and iNOS gene expressions were suppressed by cordycepin, one of the major components of *C. militaris* [35].

In the second part, the healing activity of *C. militaris* was evaluated in Wistar rats. Topical application of the ointment based on aqueous extract of *C. militaris* did not cause any skin irritation. The results of wound evolution indicate the presence of erythema and exudate in all animals from day 0, which thereafter gradually decreases over 21 days. The application of the ointment based on *C. militaris* inhibited erythema and exudates, and prevented wound odors, this reduction confirms that this mushroom could act on the inflammatory phase, as demonstrated by Chiu et al. [36] with the implication of the mycelium of *C. militaris*. As a result of this early anti-inflammatory effect of the ointment is the effective shortening of the re-epithelialization time [37]. In addition, the increase in wound contraction speed for 21 days seems to confirm the effects of the extract. The planimetric study therefore allows a direct quantitative evaluation by calculating the surface of the wound, its evolution over time, and by deducing an assessment of the quality of the granulation tissue. The evaluation of the macroscopic aspect of the wounds, which appear less inflamed with the evolution of the days, suggests that the fungi extract is able to modulate the inflammatory response. Indeed, the percentage of inhibition seems to show that the wounds treated with *C. militaris* have a better evolution than those treated with Madecassol, placebo and controls. The work of Bahramikia and Yazdanparast [38] also reported that the acceleration of the scarring process may be linked to the anti-inflammatory power by the formation of complexes neutralizing many irritants. The results of the histological study of skin biopsies of rats treated with different ointments confirm the healing effect of the ointment based on *C. militaris*. The topical application of the ointments with Madecassol and *C. militaris* (5% and 10%) induced the reconstitution of the organization of the structure of the skin and superficial scarring in the 3 groups of rats, *C. militaris* at 10% presented the best result. The moderate increase in skin tensile strength in animals after application of the ointment based on *C. militaris* may be due to the richness of collagen organized into bands.

The analysis of the mushroom material content of bioelements and organic compounds showed a source of many bioactive substances, *C. militaris* containing 32mg/g of vitamin A and 16.2 mg/g of vitamin C [39,40]. Supplementation with very high doses of vitamin A has been proposed in the event of severe wounds, but its benefit remains to be confirmed [41]. Vitamin C deficiency leads to a slowing down and poor quality of healing by reducing collagen synthesis, decreasing resistance to infections, and altering angiogenesis [42]. Vitamin C supplementation or the use of supraphysiological doses do not improve healing in non-deficient patients but could be effective in the event of aggression or severe wounds [43]. Additionally, studies have clearly shown that nanoparticles are an important platform for the treatment of skin wounds [44,45], among them Zinc, selenium and copper that *C. militaris* contains [40]. Zinc promotes cell proliferation, immunity, and resistance to apoptosis and selenium, which would play an important role in the healing of severe burns [39,46]. Copper, well known for its antimicrobial activity and angiogenesis, has also a potential role in the wound healing process by regulating the healing process at the expression level of 84 genes that are associated with angiogenesis and wound repair [47].

Additionally, Polysaccharide, important constituents of this mushroom [39], are the most effective macronutrients for collagen synthesis [41]. Recent study showed that mycelium from *in vitro* cultures and fruiting bodies of *C.militaris* contained a slightly different amount of substances, but all materials were found to be a valuable source of cordycepin, ergothioneine, indole compounds, lovastatin and bioelements [48].

5. Conclusion

This study evaluated the anti-inflammatory and wound healing potentials of the therapeutic mushroom *C.militaris* extract *in vivo*. The findings make *C. militaris* a suitable functional food for inhibiting inflammatory responses such as the regulation of proinflammatory cytokines. Moreover, this fungi may be employed in wound treatment because of its ability to accelerate wound healing. The results suggested that *C. militaris* extract has the potential to be used as topical treatment of skin injuries.

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