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# *Cedrus atlantica* (Endl.) Manetti ex Carrière Essential Oil Alleviates Pain and Inflammation with No Toxicity in Rodent

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**Abstract:** *Cedrus atlantica* (Endl.) Manetti ex Carrière is an endemic tree with spiritual value, and it was used since immemorial time in folk medicine. The present study aims to evaluate the anti-inflammatory (carrageenan-induced paw edema and formalin tests) and analgesic effects (hot plate and acetic acid writhing tests) of the cedarwood essential oil, as well as inspect any toxicity (acute toxicity), using several in vivo assays. Following the acetic acid writhing test and the hot plate test, the EO presented an excellent analgesic effect compared to the controls, especially with the dose of 50 mg/kg. Similar results were found while assessing the anti-inflammatory potential in the carrageenan-induced paw edema and formalin assays. The acute toxicity assessment and the subsequent monitoring of the animals, the biochemical analysis, and the relative organ weight, demonstrated a total safety of the EO. The GC/MS analysis of the composition revealed that the major compounds contained in this EO are beta-himachalene (51.95%), followed by alpha-himachalene (15.82%), and gamma-himachalene (12.15%). This study supports the usage of this tree EO to alleviate pain and inflammation.

**Keywords:** cedar; cedarwood; natural products; analgesia; in vivo testing; acute toxicity

## 1. Introduction

The Moroccan cedar forest, spread over an area of about 132,000 ha, produces nearly 100,000 $m^3$/year of timber logs [1]. This tree variety is a source of income for the local population, as well as the national economy [2]. This raw material constitutes an important source for the production of essential oil [3].

Essential oils, also known as volatile oils, have long been utilized in food and drinks, but they also have uses in a variety of other disciplines [4]. They are complex aromatic molecules formed from plants that are mostly made up of terpenes and other components such as aldehydes, fatty acids, phenols, ketones, esters, alcohols, nitrogen, and sulfur compounds [5]. Around 400 plant species are grown on a big commercial basis to create essential oils [6]. *Cedrus atlantica*, *C. brevifolia*, *C. deodora*, and *C. libani* are the four species

of the genus Cedrus, which belongs to the Pinaceae family. *C. atlantica* (Endl.) Manetti ex Carrière is endemic to Algeria's Tell Atlas Mountains and Morocco's Atlas Mountains (the Rif and the Middle Atlas) [7]. The essence of *Cedrus atlantica* has always been considered precious. It is used in the composition of many products, such as perfumes and some hygiene products [8]. The chemical composition of essential oils depends on several factors, such as environmental and climatic conditions, the season when the plants are harvested, storage conditions, and the quality of the oil [9,10]. The essences of several plant species have become popular in recent years, and many of their bioactive principles have recently been applied in the agri-food, pharmaceutical, medical and cosmetic fields [11,12]. Thus, the activity of essential oils against a large number of human pathogenic microorganisms has been examined in detail by several authors [13,14]. Similarly, the inhibitory effect of major constituents or total essential oil on insect pests and germs that cause food spoilage has been investigated in several studies [15]. *C. atlantica* oil has been shown to possess antimicrobial, antioxidant, analgesic, and cytotoxic activities, [16–21]. This study was undertaken to valorize the medicinal proprieties of one of Morocco's most endemic and iconic trees that has been a luxury source of wood material and perfumery over the years. In this study, well-designed procedures were performed to identify, extract, and determine the chemical composition of the essential oil obtained from the hydrodistillation of *Cedrus atlantica* wood. The medicinal proprieties were evaluated by determining the analgesic and anti-inflammatory effects with the assessment of any potential toxicity in rodents.

## 2. Results and Discussion

### 2.1. Yield of Extraction

The EO obtained in this study from *C. atlantica* wood was 3.84%. This yield was comparable with that obtained from other studies such as Fidah et al. (3.4%) [22] and Jaouadi et al. (3.51 to 5.98%) [18]. Several research in the literature have examined the EO yields of cedar wood, which vary greatly depending on the forest source and the section of the tree used, such as the work of Jaouadi et al. [18] on the EO yield from wood tar, which was found to be ranged from 8 to 11 percent.

### 2.2. Phytochemical Analysis of the Essential Oil Analysis

The phytochemical analysis revealed the presence of 13 compounds (Table 1 and Figure 1) in the *C. atlantica* EO, with a total of 98.1% of the composition identified. From the results, it appears that beta-himachalene is the major and the most abundant component in this EO with 51.95%, followed by alpha-himachalene (15.82%) and gamma-himachalene (12.15%) (Figure 2). Various studies have been carried out on to identify the chemical composition of *C. atlantica.* Strani et al. found that the major compounds of the essential oil from the Middle Atlas of Morocco were E-α-atlantone (28.75%) and β-himachalene (14.62%) [17]. β-himachalene was the major compound (23.4 to 40.4%) of the EO from seven different provenances in the Middle Atlas of Morocco [23], similar results were found in the study of Jaouadi et al., with β-himachalene as a major compound from different samples (24–44%) [18]. β-himachalene appears to be the characteristic molecule of the *C. atlantica* wood, based on the current and previous studies, with almost half the percentage of all components of the EO. Beta-himachalene belongs to the himachalane and lippifoliane sesquiterpenoids family of chemicals. Himachalane and lippifoliane sesquiterpenoids are diterpenoids having structures derived from the himachalane or lippifoliane skeletons. As a result, beta-himachalene is classified as an isoprenoid lipid molecule. Because beta-himachalene can also be present in anise [24], as well as ginger [25], it has the potential to be used as a biomarker for the ingestion of these foods.

**Table 1.** Cedarwood phytochemical components.

| | Compounds | RT | Height | Area | Start Time | End Time | Total % | Identification Confidence * |
|---|---|---|---|---|---|---|---|---|
| 1 | n-Butyl acetate | 3.201 | 210,155.846 | 3733.169 | 3.177 | 3.229 | 3.67 | 86% |
| 2 | α-Himachalene | 8.947 | 977,411.769 | 16,090.733 | 8.923 | 8.975 | 15.82 | 98% |
| 3 | γ-Himachalene | 9.124 | 595,045.667 | 12,357.828 | 9.100 | 9.172 | 12.15 | 98% |
| 4 | β-Himachalene | 9.264 | 2982,285.40 | 51,776.785 | 9.232 | 9.292 | 50.89 | 98% |
| 5 | 8-Methoxy-1-acetonaphthone | 9.328 | 55,896.667 | 853.530 | 9.304 | 9.340 | 0.84 | 83% |
| 6 | Delta-Cadinene | 9.357 | 94,491.111 | 1441.262 | 9.340 | 9.377 | 1.42 | 97% |
| 7 | α-Calacorene | 9.437 | 79,527.471 | 2472.597 | 9.401 | 9.469 | 2.43 | 80% |
| 8 | Calacorene | 9.517 | 40,319.222 | 601.620 | 9.501 | 9.537 | 0.59 | 90% |
| 9 | α-Amorphene | 10.043 | 61,618.364 | 1102.039 | 10.015 | 10.059 | 1.08 | 97% |
| 10 | Widdrene | 10.208 | 54,910.529 | 1197.305 | 10.188 | 10.256 | 1.18 | 85% |
| 11 | Trans-acrylic acid | 10.284 | 45,270.533 | 800.327 | 10.256 | 10.316 | 0.79 | 88% |
| 12 | (+)-β-Atlantone | 10.429 | 201,378.091 | 3625.321 | 10.409 | 10.453 | 3.56 | 92% |
| 13 | (+)-α-Atlantone | 10.818 | 229,762.000 | 3739.924 | 10.790 | 10.846 | 3.68 | 81% |
| Total | | | | | | | 98.1% | |

* compared to the fragmentation spectra in the WILEY7N.L database.

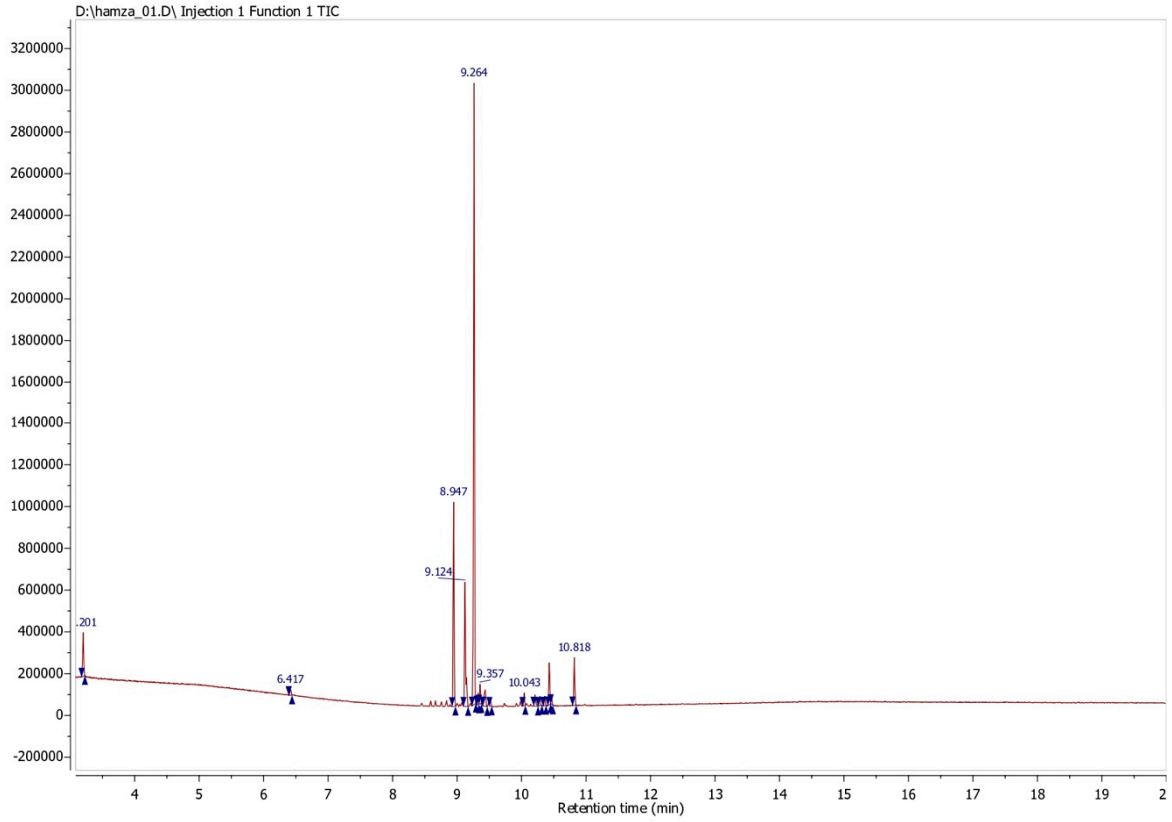



**Figure 1.** Cedarwood GC/MS chromatogram.

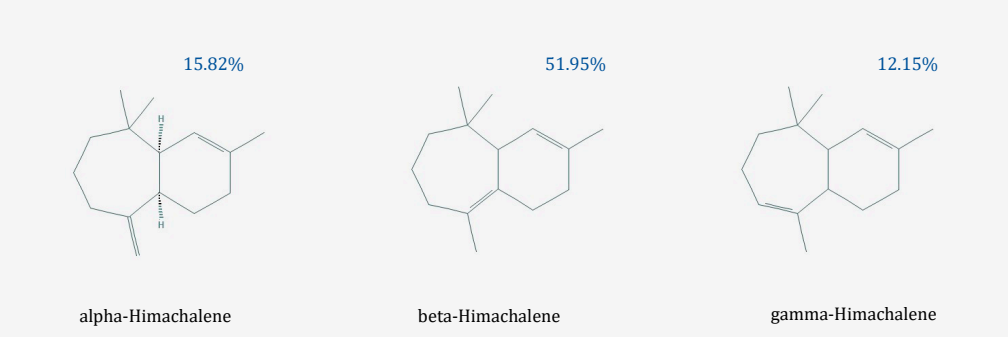



**Figure 2.** Cedar wood major compounds.

### *2.3. Anti-Nociceptive Activity Study*

#### 2.3.1. Peripheral Anti-Nociceptive Activity Assay

To assess the analgesic activity of the studied EO, the writhing test was performed. Figure 3 present the effects of diclofenac (positive control) and three concentrations of *C. Atlantica* essential oil on the percentage of inhibition of abdominal contractions.

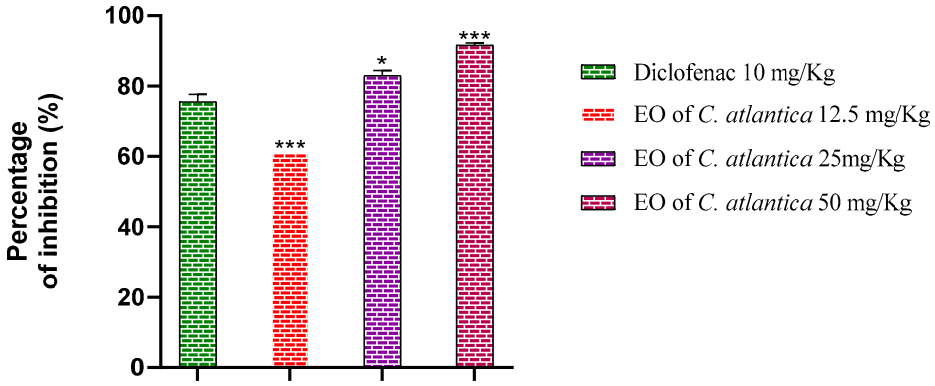



**Figure 3.** Analgesic activity of the essential oil of *C. Atlantica* by the writhing method. Values are expressed as mean ± SD ($n$ = 5). * $p < 0.05$, *** $p < 0.001$ compared to diclofenac.

After acetic acid injection to the mice, abdominal cramps are recorded after 30 min. In the presence of diclofenac at 10 mg/kg and *C. atlantica* essential oil at 12.5, 25, and 50 mg/kg, the number of abdominal cramps decreased compared to the negative control. It was also noted that the effect was dose-dependent with percentages of inhibition of contractions of 61.24%, 83.13%, and 91.65% for the doses 12.5, 25, and 50 mg/kg, respectively, compared to the positive control diclofenac 75.4%.

The doses of 25 and 50 mg/kg had a better inhibition percentage than that of the control, with the dose of 50 mg/kg being the most potent. It has been proposed that the peripheral nociceptive mechanism involved after acetic acid injection into rodents may include the action of histamine, prostaglandins PGE2 and PGE$\alpha$, serotonin, and bradykinin [26], which may be inhibited by the action of the EO components.

#### 2.3.2. Central Anti-Nociceptive Activity Assay: Hot Plate

In models of acute pain, heat is often utilized as a noxious stimulus. The dependent variable is usually the delay of the animal's reaction to the stimulation. The latency responses of treated and control animals are examined to see whether a test drug has analgesic qualities. An antinociceptive or analgesic response is defined as a substantial increase in the latency to react to the heat stimulus following a therapeutic therapy compared to a control treatment [27].

The results of the anti-nociceptive effect of *C. Atlantica* oil by the hot plate method are presented in Figure 4, which shows the maximum percentage of analgesia (MPA) of the

samples as a function of dose and time. The analysis was performed using the doses of 12.5, 25, and 50 mg/kg. As shown in Figure 4, the doses of 25 and 50 mg/kg increased analgesia and pain resistance in Swiss Albino mice. Compared with the positive control, only the dose of 50 mg/kg showed a similar analgesic effect.

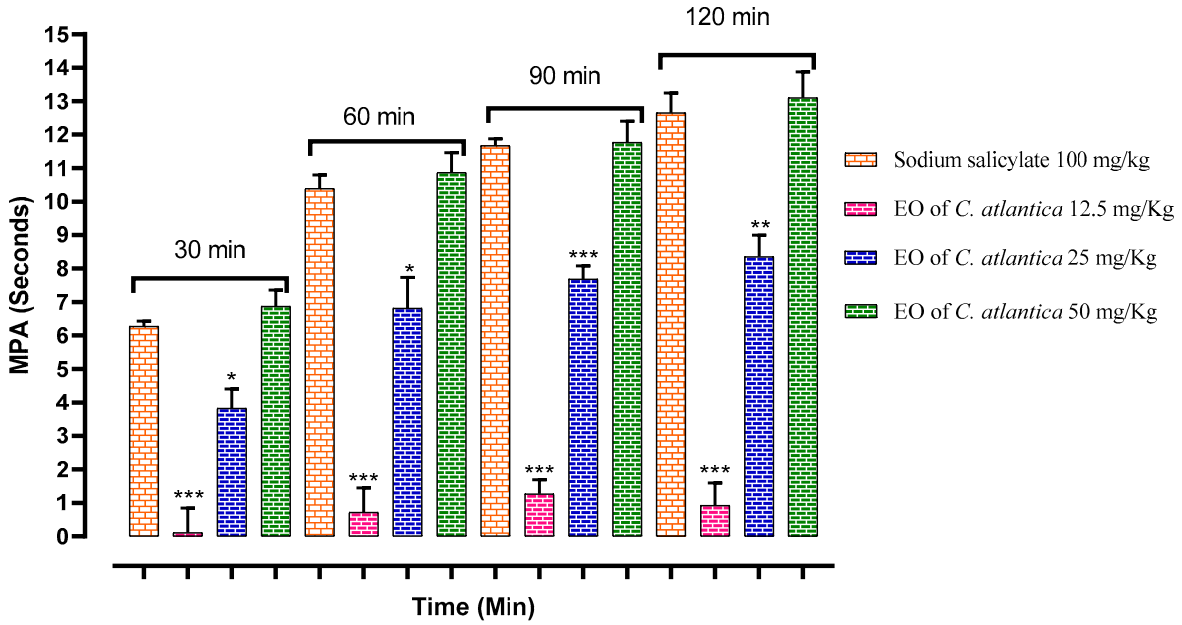



**Figure 4.** Effect of essential oil of *C. Atlantica* on the antinociceptive effect of the hot plate. Values are expressed as mean ± SD ($n = 5$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with positive control. MPA: The maximum possible analgesia (%) representing the effect of *C. Atlantica* essential oil (CDEO) versus sodium salicylate (positive control) administered to Swiss Albino mice, assessed by the hot plate method.

Studies on the analgesic and pain-relieving effect of *C. atlantica* were investigated by inhalation confirming partially some of the results obtained. In mice, Martins et al. [19] found that inhaling EO had an antihypersensitivity impact on postoperative pain induced by plantar incision surgery, and that it reduces pain behavior through the activation of opioidergic, serotonergic, noradrenergic, and dopaminergic systems, while Emer et al. [28] indicated that the inhalation of the EO In a preclinical model of postoperative pain, has an antihyperalgesic effect, probably via activation of the endocannabinoid system.

### *2.4. Anti-Inflammatory Activity Study*

#### 2.4.1. Carrageenan-Induced Paw Edema Assay

The percentages of inhibition of inflammatory edema by cedar essential oil are presented in Figure 5. A significant reduction of pain by cedar essential oil is observed at all doses from the 3rd hour of the test.

As the inhibition increase with time, it appears that the EO activity is long-lasting, and when comparing the effect of the three used doses, 12.5, 25, and 50 mg/kg, it can be said that the effect is also dose-dependent, with the dose of 50 mg/kg demonstrating the most prominent effect with an inhibition percentage of 98.36% compared to the standard anti-inflammatory drug indomethacin (20 mg/kg) percentage of 88.52% after 5 h.

Carrageenan, a substance that increases the release of inflammatory mediators, is employed in this test standardized model to examine acute inflammation. Carrageenan causes a biphasic reaction, with edema development and the release of kinins, histamine, and serotonin in the first hour after injection, and prostaglandin release 2–3 h later. Prostaglandins are the primary mediators of acute inflammation [29]. These findings show that EO has

anti-inflammatory properties in both the first and second stages of the carrageenan-induced inflammatory response.

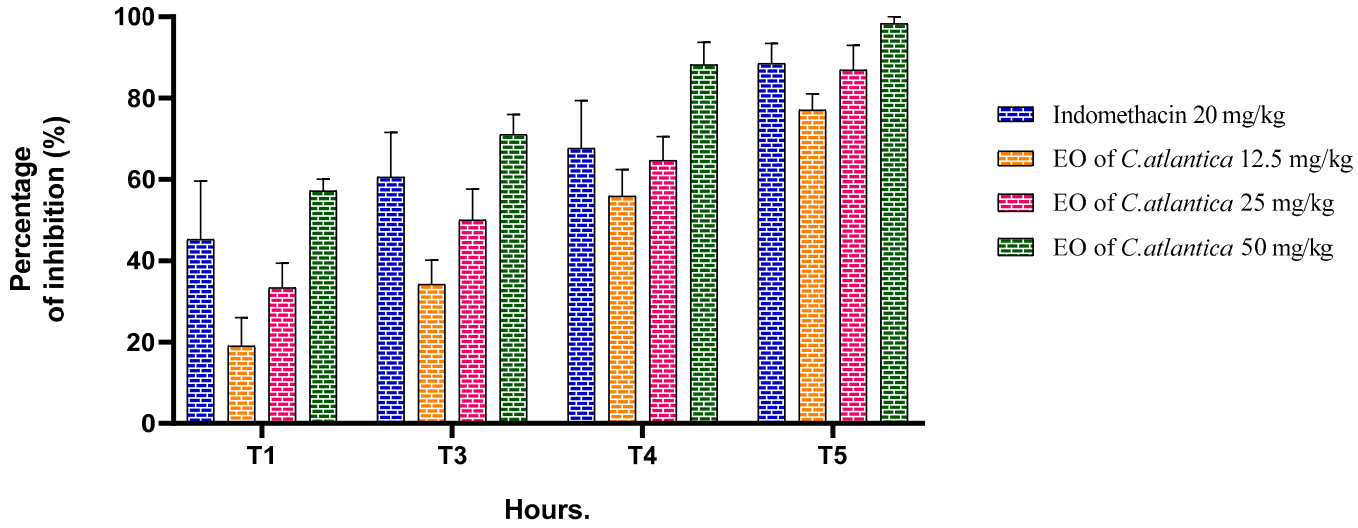



**Figure 5.** Percentage of edema inhibition. Values are expressed as mean ± SD ($n$ = 5).

2.4.2. Formalin Test

The percentages of inhibition in the formalin test are presented in Figure 6. The analysis of the results reveals that the batches treated with essential oil at the doses of 25 and 50 mg/kg show a percentage of pain inhibition comparable to that of the positive control during the first phase (0 to 5 min) and also at the second phase (15 to 30 min) while the batch treated with essential oil at the dose of 12.5 mg/kg showed a low percentage of inhibition. The results show that the EO at the dose of 50 mg/kg has a similar effect as the positive control with also a dose-dependent effect.

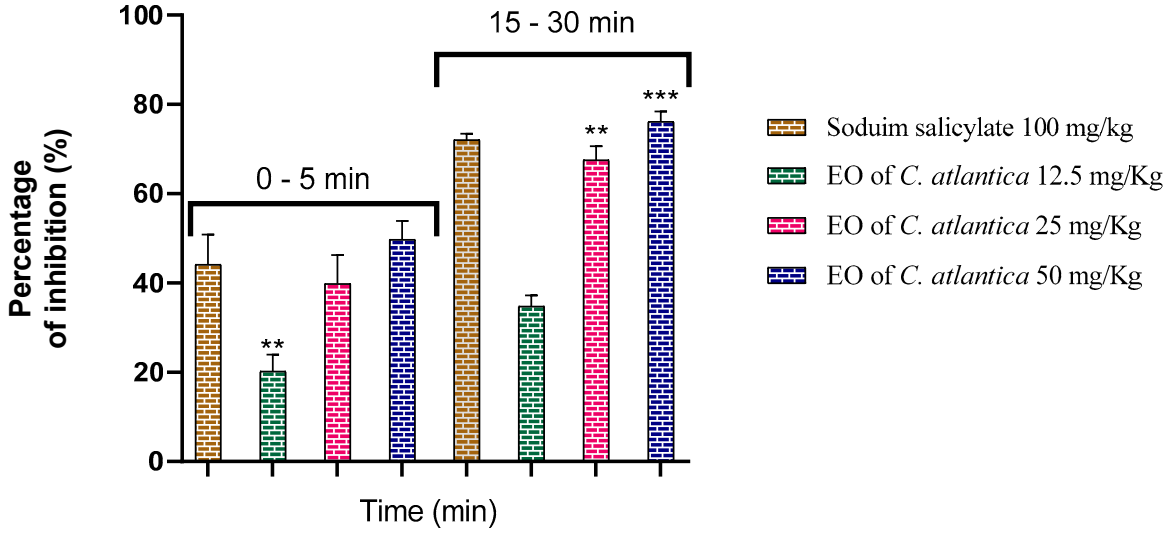



**Figure 6.** Percentage of pain inhibition. Values are expressed as mean ± SD ($n$ = 5). ** $p < 0.01$, *** $p < 0.001$ compared with positive control.

The formalin test can be pharmacologically distinguished by a biphasic response, first phase (first 10 min) and the second phase (15–20 min after the formalin injection). The first reaction is considered to be triggered by a burst of activity from pain fibers (especially C fibers), whereas the second is triggered by peripheral inflammation and may be reduced with NSAIDs. Furthermore, so-called central sensitization is thought to

have a role in the second-phase behavioral response [27]. The results show that the EO administration successfully alleviates both phases with a corresponding analgesic and anti-inflammatory potential.

Those observed anti-inflammatory potential could be attributed to some bioactive components contained in the EO, especially the major compound beta-himachalene, which a study conducted by Lenfeld et al. [30] confirmed its potential anti-inflammatory activity.

### 2.5. Acute Toxicity

The Swiss Albino mice treated with the three tested doses under acute conditions showed no signs of toxicity during the study period compared to control animals, as shown in Table 2.

**Table 2.** Behavioral parameters of the studied mice.

| | Normal Control | CDEO 12.5 mg/kg | CDEO 25 mg/kg | CDEO 50 mg/kg |
|---|---|---|---|---|
| Fur & skin | – | – | – | – |
| Eyes | – | – | – | – |
| Salivation | – | – | – | – |
| Respiration | – | – | – | – |
| Urination (color) | – | – | – | – |
| Feces consistency | – | – | – | – |
| Somatomotor activity | – | – | – | – |
| Sleep | – | – | – | – |
| Mucous membrane | – | – | – | – |
| Convulsions & tremors | – | – | – | – |
| Itching | – | – | – | – |
| Coma | – | – | – | – |
| Mortality | – | – | – | – |

–: no signs.

The body weights of control and treated mice at 12.5, 25, and 50 mg/kg are presented in Figure 7. As indicated, there was a normal increase in the body weight of treated and control mice, but no significant difference in the body weight of treated animals compared with controls.

Table 3 presented the relative organ weights of the control and dose-treated mice with 12.5, 25, and 50 mg/kg of *C. Atlantica* essential oil (CDEO) and sacrificed after 14 days of treatment. The results present are not significant when comparing it to the normal control, indicating no toxicity towards those organs.

The biochemical parameters of the liver and kidney function are presented in Figure 8. The evaluation of aspartate aminotransaminase (ASAT) shows a significant decrease in the batches treated with *C. Atlantica* essential oil at doses of 12.5 and 25 mg/kg compared to the control (Figure 8A).

Alanine aminotransaminase (ALAT) values were significantly decreased in the group treated with *C. Atlantica* essential oil at the dose of 12.5 mg/Kg compared to the control ($p < 0.05$) (Figure 8), but no significant change was observed in the other treated groups (Figure 8B).

While for the renal function, the serum creatinine concentration showed a slight elevation but was not significantly different from that of control animals (Figure 8D), and urea concentrations recorded no significant difference (Figure 8C).

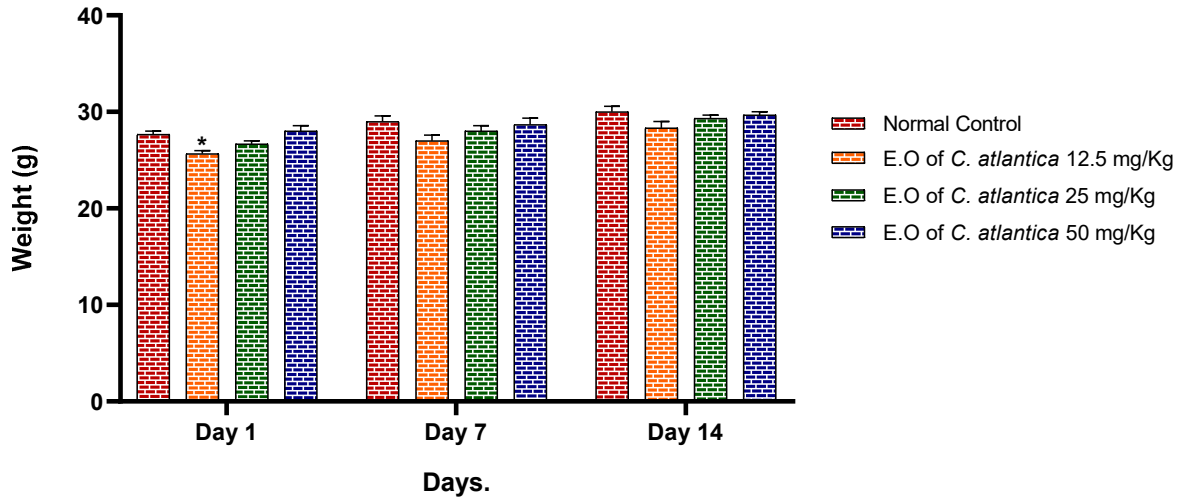



**Figure 7.** Bodyweight development of the mice. Values are expressed as mean ± SD ($n = 5$). * $p < 0.05$.

**Table 3.** Relative organ weights of the tested mice.

| | Kidneys | Liver | Heart |
|---|---|---|---|
| Normal Control | 1.457 ± 0.13 | 6.455 ± 0.062 | 0.71 ± 0.021 |
| E.O of *C. Atlantica* 12.5 mg/Kg | 1.36 ± 0.018 $^{ns}$ | 6.043 ± 0.095 $^{ns}$ | 0.68 ± 0.02 $^{ns}$ |
| E.O of *C. Atlantica* 25 mg/Kg | 1.438 ± 0.034 $^{ns}$ | 6.291 ± 0.086 $^{ns}$ | 0.702 ± 0.027 $^{ns}$ |
| E.O of *C. Atlantica* 50 mg/Kg | 1.373 ± 0.087 $^{ns}$ | 6.328 ± 0.229 $^{ns}$ | 0.734 ± 0.02 $^{ns}$ |

ns: not significant (compared to control).

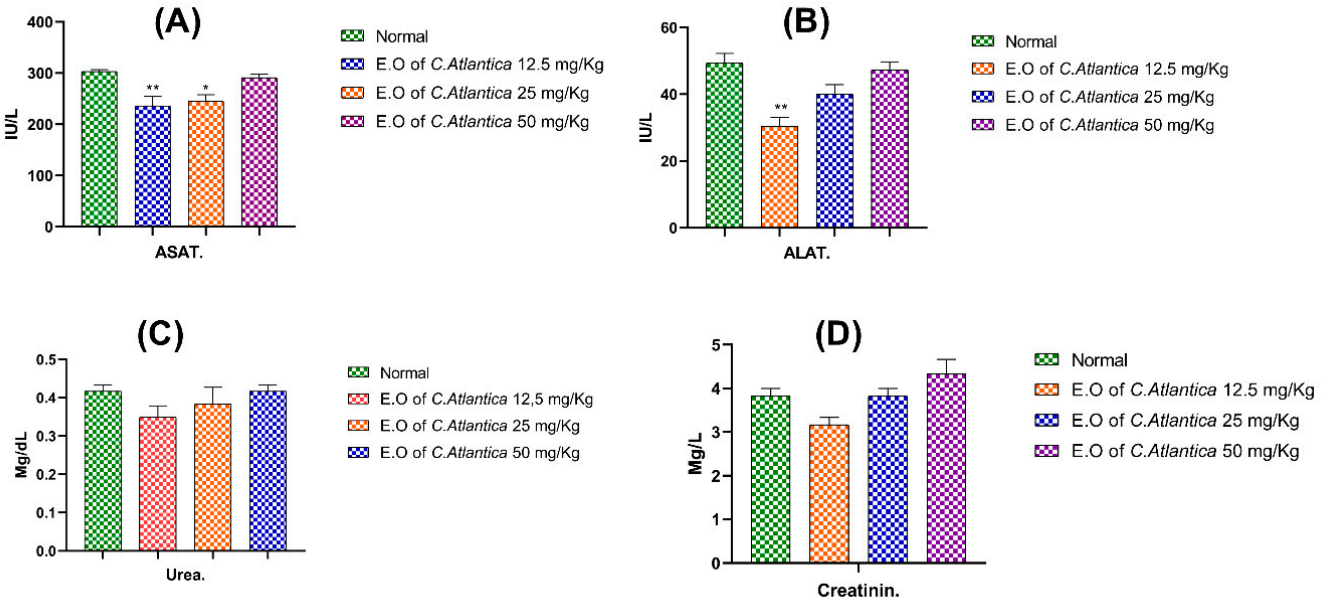



**Figure 8.** (**A**). The serum levels of ASAT; (**B**). The serum levels of ALAT; (**C**). The level of serum urea of control and treated mice (**D**). Serum creatinine level of control and treated mice. Values are expressed as mean ± SD ($n = 5$). * $p < 0.05$, ** $p < 0.01$ compared to normal control.

## 3. Materials and Methods

### *3.1. Essential Oil Preparation*

The essential oil of *Cedrus atlantica* (BPRN106) from the Middle Atlas of Morocco was extracted by hydrodistillation in a Clevenger-type apparatus. The distillation was done by

boiling 200 g of the tree wood powder in 1 L of water for 3 h. The essential oil was kept at 4 °C in the dark until further use.

### 3.2. GC Analysis: Identification of Phytochemical Compounds

The EO aliquot was prepared as follows: One mg of sample (EO weighted) was added to 1 mL of ethyl acetate. Next, 1 µL of the sample was injected in split mode for 99 analysis using a gas chromatograph (Agilent 6890 series) coupled to a mass spectrophotometer (Agilent 5973). Agilent column HP-5MS (Model number 19091S-433; 30 m long, 0.25 mm inside diameter, and 0.25 µm film thickness) was used and run in a positive mode. Helium was used as a carrier gas. The oven temperature program was set to 60–300 °C for 10 min and then maintained at 300 °C for 20 min. The detector temperature was set to 250 °C, and the injector temperature to 260 °C. Identification of the compounds was done by comparing the obtained molecule fragments (Mass spectra) with those of the Wiley 7n.L Mass Spectral Library (Wiley, New York, NY, USA).

### 3.3. Anti-Nociceptive Activity Study

#### 3.3.1. Peripheral Anti-Nociceptive Activity Assay

This study was conducted according to the method described by Bhowmick et al. [31] with some modifications. It consists in inducing an allogenic action by the injection of acetic acid (1%) to Swiss albino mice via an intraperitoneal route.

This injection induces a sensation of pain, which is manifested by a movement of stretching of the hind legs and twisting of the dorsal-abdominal musculature, called abdominal cramps. The analgesic effect is assessed by counting these cramps for 30 min after the injection of the allogenic agent [32].

Five homogeneous batches of five animals were formed, and they were fasted 16 h before testing:

- Control batch: animals in this batch received the vehicle solution (physiological water) 30 min before the injection of acetic acid (1%) intraperitoneally.
- Reference lot: animals in this lot were treated orally with Diclofenac 10 mg/kg (positive control) 30 min before intraperitoneal injection of acetic acid.
- Three test lots: the animals received the essential oil orally at three different doses, 12.5, 25, and 50 mg/kg, 30 min before the intraperitoneal injection of acetic acid.

The percentage of cramp inhibition (PI) is calculated according to the following formula:

$$PI = [(NCCo - NCTr)/NCCo] \times 100$$

NCCo = average number of contortions in the control lot.

NCTr = average number of contortions in the treated lot.

#### 3.3.2. Central Anti-Nociceptive Activity Assay: Hot Plate

In this test, mice were put on a hot plate set to 55 ± 1 °C. The mice's nociceptive reaction latency is the amount of time they take before leaping [33]. Over the course of 120 min, measurements were collected every 30 min. Mice in group 1 received 1 mL/kg of a saline solution and served as a control. Mice in group 2 received sodium salicylate (100 mg/kg (Positive Control), and groups 3, 4, and 5 received 12.5, 25, and 50 mg/kg of Cedar essential oil, respectively. The reaction time was recorded at 30, 60, 90, and 120 min after the administration of the treatments.

The maximum reaction time was set at 30 s to avoid any damage to the tissue of the paws. If the reading exceeds 30 s, it would be considered maximum analgesia.

The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = (\text{Reaction time for treatment} - \text{Reaction time for saline})/30\ s - \text{Reaction for saline} \times 100$$

### *3.4. Anti-Inflammatory Activity Study*

#### 3.4.1. Carrageenan-Induced Paw Edema Assay

In this test, the anti-inflammatory activity was assessed by the method of inhibition of paw edema induced with carrageenan [34].

A total of 25 adult male Wistar rats weighing between 150 and 220 g were used in this study (five in each group) and were maintained in a room with a constant temperature of 21 °C, a 12-h day-night cycle and access to water and food, and were fasted 15 h prior to experimentation. The circumference of the hind paw of each rat was measured prior to treatment, one hour before testing, using a digital caliper. The treatments were given by oral administration using a stomach tube.

The animals were treated with the following doses:

- Normal control: rats were treated orally with physiological water.
- Positive control: rats were treated orally with diclofenac at 10 mg/kg
- Three test lots: animals were treated with essential oil of *C. atlantica*, at the dose of 12.5, 25, and 50 mg/kg.

One hour after gavage, each animal received an injection of 0.1 mL of 1% carrageenan solution under the footpad of the paw. Measurement of paw volume was performed every hour until the sixth hour. The mean percentage increase in paw volume (PA) and the percentage of edema inhibition (PI) were calculated from the following formulas:

$$PA = [(Vt - V0)/V0] \times 100$$

$$PI = [(PATe - PATr)/PATe] \times 100$$

V0 = initial volume of the paw before induction of edema;

Vt = paw volume after administration of carrageenan administration and treatment.

PATe = percent increase in the paw volume in the paw of the control lot;

PATr = percent increase in the paw of the treated paw of the treated batch.

#### 3.4.2. Formalin Test

Subcutaneous injection of 2% formalin at the sole of the right paw 30 min after the following treatments was used to produce pain in Swiss albino mice [35]:

Group 1 was given distilled water;

Group 2 was given indomethacin (10 mg/kg);

Group 3 received a 12.5 mg/kg of essential oil;

Group 4 received a 25 mg/kg essential oil; and Group 5: received a 50 mg/kg extract.

The animals' pain response (painful paw lift) was then observed and timed for 30 min in two phases: 0–5 min early and 15–30 min late.

The pain inhibition percentages were computed as follows:

$$\% I = 100 - (MRT/MRC) * 100$$

% I = percent inhibition

MRT is an abbreviation for mean reaction time in the test group.

The mean reaction in the control group is denoted by MRC.

### *3.5. Acute Toxicity*

Twenty adult male Swiss Albino mice (weight: 25–30 g) were used for this test. They were fed a standard diet and were maintained at 24 ± 2 °C, 12-h light/dark cycle with free access to food and water. Mice were fasted for 24 h before oral administration of treatments.

Four groups of five mice each were formed and treated as follows: group 1 control was treated with distilled water, groups 2, 3, and 4 were treated with Cedarwood essential oil at doses of 12.5, 25, and 50 mg/kg, respectively.

After the administration of the essential oil at different doses, the animals were observed for 14 days to record the appearance of any of the symptoms of intoxication (i.e., piloerection, aggressiveness, mobility, alertness, stool condition, vomiting), as well as the mortality rates.

At the end of the experiment, the treated mice are sacrificed and the blood is collected to evaluate the biochemical parameters: ASAT, ALAT, urea, and creatinine, and also the relative organ weight.

### *3.6. Statistical Analysis*

The data are presented as mean ± SEM. The analyses were performed with GraphPad software Prism 6 Software (San Diego, CA, USA). All data were normally distributed. Multiple comparisons within groups were performed by repeated measures ANOVA. Statistical significance was accepted at $p < 0.05$

## 4. Conclusions

The usage of certain well-known essential oils as aromatherapy has prompted researchers to investigate and find other ones that are both safer and more effective. Many essential oils are utilized in folk medicine, and recent study suggests that more individuals should use them to maintain ancestral knowledge. The essential oil of *C. atlantica* in this study showed high potential to be used for as analgesic and anti-inflammatory. Its remarkable essence and usage as a perfume make it a more interesting combination that could work on both preventive and curative levels. The determined safety of the essential oil opens new possibilities to use this EO in the agri-food, cosmetic, and pharmaco-medical to replace synthetic and conventional analgesics and anti-inflammatories.

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