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Assessment of Acute Toxicity and Analgesic Effect of *Cedrus atlantica* (Endl.) G. Manetti ex Carrière Stem Extracts

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ABSTRACT

Cedrus atlantica is a Mediterranean medicinal plant used traditionally for the treatment of urinary tract infections and cancer. This study aims to evaluate the toxicity and analgesic properties of the stem extracts of *Cedrus atlantica*.Powdered stem of *Cedrus atlantica* was extracted successively with cyclohexane, ethyl acetate, and ethanol, in a Soxhlet apparatus, to yield extracts F1, F2, and F3, respectively. An aqueous extract (F4) was obtained by maceration of the residue at room temperature. Acute oral toxicity of the extracts was assessed in mice according to OECD guidelines. Analgesic activity of the extracts (500 mg/kg) was evaluated using the writhing and tail immersion tests, with aspirin (125 mg/kg), and morphine (0.1 mg/kg) as positive controls, respectively.Acute toxicity studies showed that *Cedrus atlantica* stem extracts have no significant toxic effects at a dose of 2000 mg/kg. In the writhing test, the aqueous extract (F4) demonstrated the highest analgesic activity with 18.33±1.55 writhes (50.66% inhibition), followed by the ethyl acetate extract (F2) with 19.33±1.77 writhes (47.97% inhibition). F2 and F4 were more effective than aspirin which caused 21.66±1.88 writhes (41.70% inhibition). In the tail immersion test, F2 and F4 showed significant central analgesic activity, both extracts significantly increased the reaction times, with F2 peaking at 5.02±0.45 seconds and F4 at 4.92±0.30 seconds, compared to morphine with reaction time of 7.70±0.18 seconds. These findings therefore suggest that *Cedrus atlantica* stem extracts are relatively safe on acute oral administration, and have potential for use as analgesic agent.

Keywords: *Cedrus atlantica*, Organic extracts, Analgesic properties, Acute toxicity, Therapeutic potential.

Introduction

Most societies have a unique ethnomedicinal history. Plants occupy a revered position and serve as a significant link between humans and nature, offering nourishment, beauty, and healing. Through a blend of traditional knowledge and scientific inquiry, the medicinal properties of plants have been uncovered, often validating the age-long wisdom that prescribed their use as remedies.[1,2]

Exploring the healing potential of plants is an intriguing and valuable endeavor as people are becoming more wary of synthetic chemicals. Plants are promising reservoirs of natural remedies, offering a fresh and safe wellspring of active ingredients. Consequently, the quest for alternative approaches becomes imperative in meeting this growing demand.[3–5]

Due to its prominent geographical position, Morocco boasts of a unique natural environment characterized by a blend of Saharan and Mediterranean climates.

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This diverse landscape nurtures a rich array of plant life, including many endemic species spanning different botanical families. Among them, one finds a plethora of rare, exceptional, and noteworthy plants that contribute to the country's botanical heritage.[6–8]

In Morocco, a diverse array of aromatic and medicinal plants thrives across different landscapes, showcasing interesting therapeutic properties. Empirical evidence attests to their effectiveness in offering relief, if not outright cure for many diseases.[9,10] Remarkably, the World Health Organization (2003) notes that about 80% of people worldwide turn to traditional remedies for their basic healthcare needs. This reliance underscores the vast pharmacological potential present in nature, with over 20,000 plant species contributing to the pharmacopeia, accounting for more than half of the pharmaceutical products available globally.[11]

The present study is focused on *Cedrus atlantica* (Endl.) G. Manetti ex Carrière, a species that occupies a crucial ecological niche within the forests of northern Africa. The plant belongs to the Pinaceae family, Abieteae subfamily, and *Cedrus* genus, it is esteemed for its dual significance in both environmental and commercial contexts, particularly in the Mediterranean uplands of Morocco. *Cedrus atlantica* is recognized in traditional medicine for its diverse therapeutic properties, serving as a versatile remedy for various ailments. It has been utilized to address skin problems in animals and to combat parasitic infections,[14] alongside its potential applications in cancer therapy,[15] cellulite reduction,[16] and stress management. Notably, it has also been recommended for anxiety relief and tension alleviation.[17] Its well-documented anti-inflammatory properties extend from treating specific conditions such as hay fever,[18] to promoting

mucolysis, managing catarrh, and addressing chronic bronchitis.[19] Additionally, it shows promise in treating cystitis and urinary tract infections.[20] Beyond medicinal uses, the plant *Cedrus atlantica* contributes to enhancing both hair and skin health, effectively addressing conditions such as oily skin, dandruff, and acne.[21] Through phytochemical analysis, various bioactive compounds have been identified in *C. atlantica*, including sesquiterpene hydrocarbons, monoterpene hydrocarbons, and oxygenated monoterpenes.[22–26]

Certainly, scientific research has substantiated the traditional uses of this plant. Literature survey reveal a spectrum of biological activities associated with the plant, ranging from antibacterial,[26,27] analgesic,[28] anticancer,[29,30] antifungal,[31] and antiparasitic [32,33] to antioxidant properties.[34,35]

Considering the aforementioned benefits of *C. atlantica*, the main goal of this work is to evaluate the analgesic activity of the sten extracts, and to assess the safety profile of organic solvent fractions of *C. atlantica* from Morocco through acute toxicity evaluation.

Materials and Methods

Chemicals

The chemicals used were of analytical grades. Specifically, cyclohexane, ethyl acetate, ethanol, and acetic acid were products of Sigma Aldrich and Solvachim chemicals.

Collection and identification of plant materials

Cedar stems were harvested in October 2021 from the Atlas Mountains in the Ifrane region of Morocco, precisely at geographical coordinates 33° 29' 54.2" N and 5° 08' 06.2" W. The plant materials were identified and authenticated at the Scientific Center in Rabat, an herbarium specimen was prepared, and deposited at the institution's herbarium, and was designated the voucher specimen number RAB114017.

Preparation of extracts

Cedar stems were air-dried in the shade at room temperature. Subsequently, they were finely ground into a powder with particle sizes smaller than 25 µm.

The powdered plant material (50 g) was placed into a cotton cellulose cartridge. The material was then subjected to successive extraction using 400 mL of various solvents (cyclohexane F1, ethyl acetate F2, and ethanol F3, in that order) in a Soxhlet apparatus. Extraction with cyclohexane lasted for 6 h, while ethyl acetate and ethanol extractions lasted 8 h each. The completion of the extraction process was signaled by the appearance of colorless solvents in the siphon tube. Thereafter, the residual plant material was macerated with 1000 mL of distilled water (F4) at room temperature for 8 h. The resulting crude extracts were filtered using Whatman filter paper and concentrated using a rotary evaporator (GREATWALL R-1001 Rotavapor WB-2000 Water bath, China) under reduced pressure. The bath temperature was maintained at 35 - 40°C, with rotation at a speed of 120 rpm. Finally, the concentrated extracts were stored at 4°C until required.[36]

Animals

Female Wistar rats weighing between 160 and 240 g, as well as Swiss albino mice weighing between 20 and 30 g, were obtained from the animal facility at the Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco. The animals were kept under controlled environmental conditions at a temperature of 22 ± 2°C, with a light-dark cycle of 14 h and 10 h, respectively. They had unrestricted access to food and water throughout the duration of the study.

All experimental protocols adhered strictly to the "Principles of Laboratory Animal Care" and were executed in line with the "Guide for the Care and Use of Laboratory Animals" by the National Academy of Sciences.

Acute toxicity evaluation

An acute oral toxicity assessment of *C. atlantica* stem extracts was conducted following the guidelines outlined by the Organization for Economic Cooperation and Development (OECD 423). Three non-pregnant, nulliparous female mice weighing between 20 and 30 g were fasted for 4 h. Each mouse was then placed individually in sterile polypropylene cages with free access to water. Subsequently, the *C. atlantica* stem extracts were administered orally to each mouse using an esophageal probe at a dose of 2000 mg/kg. The selection of extract doses was guided by the substances' toxic potential. Following the administration of the extracts, the animals were monitored initially for 30 minutes, and then for 14 days for signs of toxicity such as change in body weight, salivation, lethargy, convulsions, diarrhea, sleepiness, coma, and death.

Evaluation of analgesic effect

Acetic Acid-Induced Writhing Test

The acetic acid-induced writhing test was performed according to the method described by Koster et al. (1959)[37] and modified by El-guourrami et al. (2023).[38] The mice were weighed, and then randomly divided into six groups (I – VI), each comprising five mice. Group I served as the control group, and had no treatment. Groups II – V were administered 500 mg/kg per oral dose of F1, F2, F3, and F4, respectively. Group VI was admistered aspirin at a dose of 125 mg/kg orally. Thirty minutes post administration of the extracts, each mouse received an intraperitoneal injection of 3% (v/v) acetic acid at a dose of 3.75 mL/kg body weight. After 10 min of acetic acid administration, the occurrence of abdominal writhing in each mouse was monitored for 10 min. The percentage inhibition of abdominal writhing was calculated using the formula below:

$$\% \ \boldsymbol{Inhibition} = \mathbf{1} - \frac{\boldsymbol{Average\ writhes\ of\ the\ treatment\ group}}{\boldsymbol{Average\ writhes\ of\ the\ control\ group}} \times \mathbf{100} \quad (1)$$

Tail Immersion Test

The tail immersion test followed the procedure described by Sewell *and* Spencer (1976).[39] Female rats weighing between 160 and 240 g were divided into six groups of five animals each. Group I was used as the control and received no treatment, groups II – V received cedar stem extracts (F1, F2, F3, and F4) at a dose of 500 mg/kg each, while group VI received the reference drug morphine at a dose of 0.1 mg/kg. Rats' tail segments measuring 6 cm from the tip were submerged in a water bath maintained at 55 ± 0.5°C. The duration from tail immersion to tail response was measured at 0, 30, 60, 90, and 120 min following the administration of morphine or extracts, using a digital stopwatch. A contact time of 10 sec was allowed to prevent harm to the animals.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism v8 software. Data were presented as mean ± standard deviation of triplicates measurements. The data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine the differences between the mean values. Statistical difference between means was established at $p \leq 0.05$.

Results and Discussion

Acute toxicity of C. atlantica stem extracts

Evaluating the toxicity of a substance intended for therapeutic purposes is undoubtedly a critical aspect of the drug discovery process. Typically, short-term toxicity assessment serves as the initial *in vivo* test model during preclinical drug development.[40]

The results of the acute toxicity evaluation of *C. atlantica* stem extracts revealed no sign of toxicity. Common clinical indicators of acute toxicity, such as convulsions, excessive salivation, diarrhea, hypnosis, and coma, were notably absent in the animals during the initial 30 minutes observation period following the administration of the extract and throughout the subsequent 14 days monitoring period. Furthermore, no morbidity or mortality was observed in the animals, suggesting that the plant extracts have no severe toxic effects. These observations imply that the extracts of *C. atlantica*, at the specified dose, do not present significant acute toxicity risks to the test animals, with the median lethal dose (LD_{50}) exceeding 2,000 mg/kg. Consequently, according to the criteria outlined in OECD No.423, the

extracts of *C. atlantica* were deemed non-toxic at a single oral dose of 2,000 mg/kg. However, it is crucial to know that these results are specific for the conditions of this particular study and should not be extrapolated to different doses or other animal species without further investigation. Figure 1 illustrates the body weight of animals treated with extracts of *C. atlantica* at 2,000 mg/kg during the acute toxicity study. There was no significant change in the body weight of the mice, which suggest that the stem extracts of *C. atlantica* have no adverse effect on the growth of the animals. It is important to note that careful monitoring and additional studies are necessary to evaluate the long-term effects and safety of these extracts. To the best of our knowledge, there are no scientific reports on the toxicity evaluation of *C. atlantica* extracts. Therefore, this study represents the first study on the assessment of the safety of *C. atlantica* stem extracts.

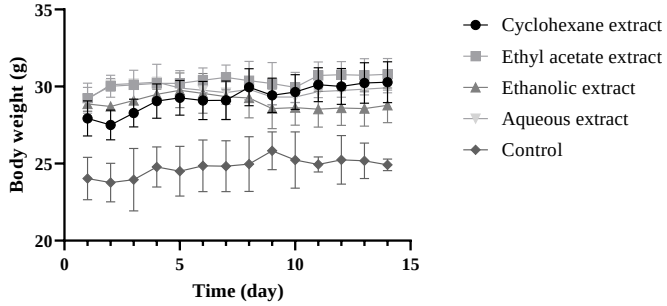

Figure 1: Body weight of animals administered *C. atlantica* extracts at 2000 mg/kg in the acute toxicity study.

Analgesic activity of C. atlantica stem extracts

Table 1 show the analgesic activity of *C. atlantica* stem extracts and that of the standard drug acetylsalicylic acid (aspirin) as measured by acid-induced writhing in mice, a commonly used model for evaluating analgesic effect. Each treatment group received a dose of 500 mg/kg of one of four extracts: cyclohexane (F1), ethyl acetate (F2), ethanol (F3), and aqueous (F4). Aspirin, which was used as a reference drug, was administered at a dose of 125 mg/kg, while the negative control group received no treatment. Writhing, which is indicative of pain, and the percentage inhibition of writhing which is a reflection of analgesic efficacy of the extracts, were recorded. The negative control group showed an average of 37.15 ± 1.77 writhes, serving as a reference for pain response. The aqueous extract (F4) demonstrated the strongest analgesic activity, with an average of 18.33 ± 1.55 writhes and a percentage inhibition of 50.66%. This was followed by the ethyl acetate extract (F2) which showed significant pain relief, with an average of 19.33 ± 1.77 writhes and a percentage inhibition of 47.97%. The ethanol (F3) and cyclohexane (F1) extracts displayed an average of 21.33 ± 1.11 and 30.66 ± 2.88 writhes, with percentage inhibitions of 42.58% and 17.47%, respectively. Aspirin, which was used as the positive control, resulted in an average of 21.66 ± 1.88 writhes, with a percentage inhibition of 41.70%. Although, there were no statistically significant differences in the analgesic activity of F2, F3, F4, and aspirin ($p > 0.05$), the analgesic effect of the aqueous and ethyl acetate extracts markedly surpassed that of aspirin, suggesting potent analgesic properties of these extracts.

The central analgesic potential of the extracts, particularly the central pain mechanisms, was further examined using the tail immersion test. Table 2 presents the effect of the four extracts of *C. atlantica* stem on the average reaction time, as measured by the tail immersion test in Wistar rats. The study assessed the reaction times at five different time intervals (0, 30, 60, 90, and 120 minutes) after administration of the extracts or morphine. Each extract dose was 500 mg/kg, while morphine was administered intraperitoneally at 0.1 mg/kg. The control group received no treatment. The initial reaction times (0 min) were similar across all groups, about 2.3 to 2.5 seconds, with no significant difference ($p > 0.05$), thus establishing a baseline. The morphine group showed a substantial increase in reaction time, peaking at 7.70 ± 0.18 seconds at 120 minutes, demonstrating its strong analgesic effect. Among the extracts, those of ethyl acetate (F2) and aqueous extract (F4) revealed notable analgesic effects. F2 increased reaction times to 5.02 ± 0.45 seconds at 30 minutes and maintained higher values compared to the baseline up to 120 minutes. F4 also showed a significant increase, peaking at 4.92 ± 0.30 seconds at 30 minutes and remaining elevated up to 120 minutes. The ethanol extract (F3) exhibited the most significant effect after 60 minutes with a reaction time of 5.74 ± 0.02 seconds, which gradually decreased thereafter. The cyclohexane extract (F1) showed the least analgesic effect among the extracts, with a maximum reaction time of 3.54 ± 0.03 seconds at 30 minutes, and slightly decreased over time.

The data indicated that ethyl acetate (F2) and aqueous (F4) extracts of *C. atlantica* stem significantly improved nociceptive response times, albeit not to the same extent as morphine. This could be attributed to the presence of phenolic compounds in the plant, particularly flavonoids,[14] suggesting that they could serve as an effective alternative in pain management.

The analgesic activity of *C. atlantica* extracts has not been evaluated to date. Therefore, this current study represents the first exploration of the analgesic potential of the plant. Additionally, previous publications have described the analgesic activity of various plants within the *Cedrus* genus. For instance, in a study by Karrat *et al*. (2022),[41] the analgesic effects of ethanol extracts of *Cedrus libani* from Syria were assessed using a formalin test at a dose of 30 mg/kg and a tail flick test using a gel containing 2% (w/v) ethanol extract. Positive controls such as diclofenac sodium, 1% diclofenac gel, and 2% lidocaine gel were utilized. Results indicated that the extracts significantly reduced paw licking time and inhibited paw edema in the formalin test, outperforming the positive controls. Moreover, in the tail flick test, the plant extract gel exhibited greater efficacy than diclofenac gel when the maximum possible effect (MPE) was calculated. It was suggested that the observed effects could be attributed to phytochemical compounds such as flavonoids and tannins.

This study explores the analgesic potential of *C. atlantica* extracts using experimental models such as writhing and tail immersion tests. Ethyl acetate and aqueous extracts demonstrated significant analgesic effects, comparable to or exceeding that of aspirin for peripheral analgesia, and notable central analgesic activity, but less potent than morphine. This observation suggests potential modulation of opioid receptors or inhibition of pain signaling pathways. The strategic use of various solvents (cyclohexane, ethyl acetate, ethanol, and water) facilitated the exploration of *C. atlantica* extract chemical diversity. Each solvent extracted distinct classes of phytochemicals with varying polarities, thereby influencing their bioactivity. For instance, ethyl acetate extracts exhibited intense analgesic activity, implicating lipophilic compounds such as flavonoids, while aqueous extracts showed significant analgesic activity, possibly attributed to hydrophilic compounds or synergistic interactions among constituents.

Conclusion

The present study highlighted the promising analgesic potential of *C. atlantica* stem, with the extracts demonstrating efficacy comparable to morphine and a notable absence of toxic effect at tested doses. These findings suggest *C. atlantica* as a viable alternative for pain management, necessitating further investigations in clinical settings. The observed effects could pave the way for future study aimed at elucidating the underlying mechanisms and optimizing therapeutic applications of *C. atlantica* extracts in alleviating pain.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 1: Analgesic effect of *C. atlantica* stem extract and Aspirin on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	Number of writhes	Percentage Inhibition (%)
F1	500	30.66 ± 2.88^{a}	17.47
F2	500	19.33 ± 1.77^{b}	47.97
F3	500	21.33 ± 1.11^{b}	42.58
F4	500	18.33 ± 1.55^{b}	50.66
Aspirin	125	21.66 ± 1.88^{b}	41.70
Negative Control	-	37.15 ± 1.77^{c}	-

Data represent the mean ± standard deviation of five independent experiments. Values in the same column with different superscript letters indicate significant differences (p-value < 0.05). F1: Cyclohexane extract; F2: Ethyl acetate extract; F3: Ethanol extract; F4: Aqueous extract.

Table 2: Analgesic impact of *C. atlantica* Stem extracts and morphine on nociceptive responses in the tail immersion test

Treatment	Dose (mg/kg)	Reaction time in seconds				
		0 min	30 min	60 min	90 min	120 min
F1	500	2.37 ± 0.12^{a}	3.54 ± 0.03^{a}	3.51 ± 0.52^{a}	3.33 ± 0.27^{a}	3.27 ± 0.09^{a}
F2	500	2.44 ± 0.21^{a}	5.02 ± 0.45^{b}	4.43 ± 0.03^{b}	3.82 ± 0.36^{ab}	3.40 ± 0.17^{a}
F3	500	2.25 ± 0.04^{a}	3.86 ± 0.21^{a}	5.74 ± 0.02^{c}	4.02 ± 0.02^{ab}	3.63 ± 0.34^{a}
F4	500	2.32 ± 0.19^{a}	4.92 ± 0.30^{b}	4.48 ± 0.12^{b}	4.26 ± 0.17^{b}	3.87 ± 0.32^{a}
Morphine	0.1	2.53 ± 0.43^{a}	6.46 ± 0.13^{c}	6.75 ± 0.12^{d}	7.25 ± 0.15^{c}	7.70 ± 0.18^{b}
Negative control	-	2.35 ± 0.32^{a}	2.62 ± 0.39^{d}	2.63 ± 0.47^{e}	2.33 ± 0.39^{d}	2.03 ± 0.31^{c}

Data represent the mean ± standard deviation of five independent experiments. Values in the same column with different superscript letters indicate significant differences (p-value < 0.05). F1: Cyclohexane extract; F2: Ethyl acetate extract; F3: Ethanol extract; F4: Aqueous extract.

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