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Original Article

Exploring the *In vitro* and *In vivo* therapeutic efficacy of *Juniperus oxycedrus* Cade oil: Antioxidant, anti-inflammatory, and anti-asthmatic effects in an allergic asthma model

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Article Info

Abstract

This investigation aimed to explore the antioxidant, anti-inflammatory effects of Cade oil and its efficacy within a Wistar allergic asthma model. The antioxidant activity was assessed through various in vitro tests using chain-breaking antioxidant effects (radical scavenging and reducing abilities assays). In vivo experiments involved Wistar rats categorized into four groups: negative control group, Ovalbumin-sensitised/challenged group, Cade oil-treated group, and Ovalbumin-sensitised/challenged Cade oil-treated group. These experiments aimed to evaluate oxidative stress parameters in the lungs and erythrocytes. The results indicated that the Cade oil exhibited significant antioxidant capabilities, evidenced by its radical scavenging activity against DPPH, ABTS, and Galvinoxyl radicals, with IC_{50} values ranging from 21.92 to 24.44 µg/mL. Besides, the reducing abilities methods showed A0,5 value ranging from 11.51 to 30.40 µg/mL for reducing power, Cupric ion reducing antioxidant capacity, and O-phenanthroline assays. Additionally, the IC_{50} value for β-carotene scavenging was found to be (8.2 ± 0.25 µg/ml). Analysis revealed high levels of polyphenols and flavonoids in Cade oil, indicating rich polyphenol (275.21 ± 3.14 mg GAE/g DW) and flavonoid (28.23 ± 1.91 µg QE/mg) content. In vivo findings highlighted Cade oil's efficacy in reducing inflammatory cell recruitment, enhancing antioxidant status, reducing lipid peroxidation, and improving histopathological alterations within the allergic asthma model. These results demonstrated that Cade oil has a potent antioxidant, anti-inflammatory, and anti-asthmatic properties, suggesting its potential therapeutic application in asthma treatment.

Keywords: Allergic asthma model, Antioxidant activity, Cade oil, *Juniperus oxycedrus*, Lung inflammation, Oxidative stress.

1. Introduction

Asthma is a complex, heterogeneous condition affecting the conducting respiratory tract, characterized by chronic inflammation, reversible airflow blockage, heightened bronchial hyper-responsiveness and oxidative stress [1, 2]. Despite advances in effective treatment during the last decades, asthma currently affects around 0.3 billion people worldwide, incurring a heavy burden on public health, as noted by the World Health Organization [3]. Recent studies have emphasized the significant role of oxidative stress in exacerbating asthma symptoms, including airway inflammation, decreasing lung function, and tissue damage. Chronic inflammation in the respiratory tract is recognized as the fundamental pathological feature of asthma, exacerbated by an increase in oxidative stress-induced inflammatory mediators [2]. The lungs, due to their

exposure to environmental and endogenous antioxidants, are particularly vulnerable to oxidative damage, contributing to an imbalance arises between the generation and accumulation of reactive species (ROS/RNS) and the antioxidant defence systems [4]. This imbalance intensifies the inflammatory process, highlighting the need for treatments that can effectively modulate these responses with minimal side effects [5].

Natural compounds, including flavonoids and polyphenols, have shown promise in asthma treatment due to their antioxidant and immunomodulatory properties [5]. Among these, *Juniperus oxycedrus* L. known locally as (Tagga) and found in the Mediterranean region, including parts of Algeria [6], is reported to possess numerous biological activities, essentially antioxidant [6] and anti-inflammatory properties [7]. Cade oil, sesquiterpene oil derived

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from the destruction distillation of *Juniperus oxycedrus* branches and wood, has been traditionally exploited in Turkish folk medicine to treat various ailments, including asthma, cold, cough, rheumatism and skin conditions [8].

However, despite the several known benefits, many studies described in the literature reported the adverse effects and incidents of poisoning arising from the randomly excessive consumption of Cade oil [9]. These incidents highlight the importance of a balanced approach, based on rigorous scientific evidence, to validate the traditional uses of Cade oil [9]. Although many traditional medicines exhibit inherent toxicity, they are considered safe when used in controlled dosages [10].

The main focus of this study is to examine the *in vitro* antioxidant activity and *in vivo* antioxidant, anti-inflammatory and anti-asthmatic effects of Cade oil in a Wistar rat model of allergic respiratory disorder by Cade oil administration.

2. Materials and methods
2.1 Plant material
The traditional production of Cade oil is based on a direct steam distillation of the woods and branches of *Juniperus oxycedrus*, collected from the Eastern Mediterranean coast of Algeria and marketed by local herbalists.

2.2 Chemicals
All chemicals and reagents used in the in vitro experiments were purchased from both Sigma Chemicals Co and Biochem Chemopharma. Chicken egg albumin (Ovalbumin, OA, grade II), Bovine serum albumin (BSA) and each chemical utilised in the in vivo study were purchased from Sigma Chemical Co (St. Louis, Mo).

2.3 Determination of total bioactive content
2.3.1 Determination of the total phenolic content (TPC)
Spectrophotometric analysis was carried out using the Folin-Ciocalteu reagent, according to a modified procedure by Singleton and Rossi [11].The absorbance against a blank was measured at 765nm. The total phenolic content was expressed as micrograms of gallic acid equivalents per milligrams of extract (μg GAE/mg).

2.3.2 Determination of the total flavonoid content (TFC)
A modified method based on the procedure described by Topçu et al. [12] was employed. The absorbance was measured at 415nm. The total flavonoid content was expressed as micrograms quercetin equivalents per milligram of extract (μg QE/mg).

2.4 In vitro antioxidant activity determination
2.4.1 Free Radical Scavenger Activity by DPPH
The capacity of Cade oil to inhibit the free radical DPPH (2,2-diphenyl-1- picrylhydrazyl) was determined using the method reported by Meziant et al. [13]. The results were compared to reference antioxidant standards (BHA and BHT and Ascorbic acid). The findings were presented regarding the concentration required for 50% inhibition (IC50), expressed in μg/mL.

2.4.2 ABTS scavenging activity
The ABTS+ radical cation decolourisation assay was employed to carry out the ABTS scavenging activity, according to the method described by Meziant et al. [13].

2.4.3 β-carotene bleaching inhibition
The β-carotene/linoleic acid assay was assessed following the protocol described by Lekouaghet et al. [14]. and the results were given as 50% inhibition concentration (IC_{50}) (μg/mL).

2.4.4 Reducing power assay
The ferric-reducing power was assessed according to Meziant et al. [13]. The results were presented as $A_{0.50}$ (0.50 absorbance), indicating the concentration level required to achieve an absorbance of 0.5.

2.4.5 Phenanthroline assay
Phenanthroline antioxidant activity was performed in a 96-well microplate following the method detailed by Bakhouche et al. [15]. The absorbance of the resulting solution was determined at 510 nm, with BHT and BHT utilised as standards.

2.4.6 Cupric reducing antioxidant capacity (CUPRAC)
The evaluation of the reducing antioxidant capacity was assessed by the (CUPRAC) assay following the method described by Lekouaghet et al. [14]. Results were expressed as $A_{0.5}$. BHA, BHT and Ascorbic acid were used as antioxidant standards.

2.4.7 Galvinoxyl free radicals scavenging assay (GOR)
The extract impact on the Galvinoxyl free radicals was estimated according to Bakhouche et al. [15]. The absorbance was measured at 428 nm. Galvinoxyl solution in methanol was used as a control, while BHA and BHT served as standards in this study. The results were represented as EC_{50} (μg/mL), indicating the concentration at which 50% absorbance intensity was observed.

2.5 Determination of the in vivo antioxidant activity
2.5.1 Animals
Twenty-four male Wistar rats weighing between (240 and 250g) were utilised. The rats were obtained from the Pasteur Institute of Algiers (Algiers, Algeria) and provided unrestricted access to water and the standard feed supplied by the ONAB of Bejaia (Algeria) throughout the experiment. The animals were housed under natural photoperiod conditions, with an average temperature maintained at 21-25°C.

The methodologies applied in this study received approval from the Ethical Committee of the Directorate General for Scientific Research and Technological Development, which operates under the auspices of the Algerian Ministry of Higher Education and Scientific Research. This endorsement was formalised with the issuance of license number PNR/SF 08/2012, ensuring adherence to ethical standards in the research process.

2.5.2 Experimental group design
Twenty-four rats were divided into four groups, with six in each group. The control group (CTL), which received no treatment, was considered as a negative control group. Rats challenged and sensitised with Ovalbumin are included in the asthma group (OVA), which is regarded as the positive control. The treated Group with Cade oil (CO) received oral administration of Cade oil (1mL/kg) for 10 days following the experimental protocol. Asthma treated Group (OVA+CO) corresponding to Ovalbumin-challen-

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ged rats, treated for 10 days with Cade oil (1 mL/kg).

2.5.3 Sensitisation and airway challenge

In the OVA and OVA+CO groups, sensitisation was performed on days 0 and 14 by intraperitoneal injections of Ovalbumin (Ovalbumin, OA, grade II) (1 mg/mL) along with alum (1 mg/mL in saline solution) as an adjuvant. Subsequently, on the 21st, 22nd, and 23rd days, the rats in these groups were subjected to a 30-minute OVA inhalation challenge (5 mg/mL) using an OMRON nebuliser (NE-C29-E). On the other hand, the CTL and CO groups were injected and challenged with saline on the same dates. The methodologies described by Moura et al. [16] and Yang et al. [17] were adopted. The samples collected for analysis were namely lung tissue, blood and bronchoalveolar fluid samples.

2.5.4 Samples preparation

Animals were sacrificed twenty-four hours following the final aerosol challenge, by cervical decapitation. The Blood samples were collected in plain vials and heparin tubes to obtain serum and plasma, respectively. Blood samples in plain vials were centrifuged (3000 rpm for 15 minutes at 4°C) and then preserved at -20°C. The erythrocytes in the blood sediment were centrifuged after being twice suspended in PBS (pH 7.4), first at 3000 g for 15 minutes at 4°C and then at 4000 g for 30 minutes. Then, the erythrocyte solution was collected in Eppendorf tubes and stored at 20°C. The trachea of each rat was cannulated, and the lungs of each rat underwent three successive lavages with PBS (2mL). This lavage fluid was centrifuged at 4000 x g for 10 min. Eppendorf tubes containing Broncho Alveolar Lavage Fluid (BALF) were stored at −20 °C. The cell pellet was vortexed and suspended in 500 μL of PBS for cell counting using an improved Neubauer hemocytometer (Full Automatic Blood Cell Counter MODEL PCE-210N). Afterwards, Lung tissues were promptly extracted, rinsed with a 0.9% saline solution, and then weighed after carefully removing adjacent connective tissues. Approximately 1g of the lung was homogenised in a 2:1 volume of PBS (Tris 50mM, NaCl 150mM, pH 7.4) under an ice-cold condition. After centrifugation at 3000 × g for 35 minutes at 4°C, the resulting supernatants were conserved at -20°C.

2.5.5 Lipid peroxidation levels measurement

Lipid peroxidation levels in both lungs and erythrocytes were evaluated using Malondialdehyde (MDA) as a biomarker. MDA, a final product of lipid peroxidation, interacted with thiobarbituric acid (TBA) to form a red complex known as TBARS. The concentration of TBARS was quantified by measuring its absorbance at 530, following the method described by Esterbauer et al. [18].

2.5.6 Reduced glutathione (GSH) levels measurement

GSH levels in both lung and erythrocytes were assessed using a colourimetric method following Weckbecker and Corry [19]. In this method, SH groups of glutathione reduced DTNB (5,5'-dithiobis-(2-nitrobenzoic acid), producing a characteristic yellow colour. The absorbance at 412 nm was recorded, and the total GSH concentration was presented in nmol GSH/mg protein.

2.5.7 Determination of antioxidant enzyme activities

In both lung tissue and erythrocytes, the roles of antioxidant enzymes were determined through glutathione peroxidase (GPx) and catalase (CAT) activities. The procedure outlined by Flohé and Günzler [20] served as the basis for measuring GPx activity; this implies that the hydrogen peroxide (H_2O_2) was reduced to glutathione disulfide (GSSG) in the presence of reduced glutathione (GSH). The specific GPx activity was quantified at 420 nm and presented as mmol GSH/mg protein. In addition, catalase activity was assessed by measuring the ability of the enzyme to catalyse the degradation of hydrogen peroxide, which led to a decline in absorbance, following the method reported by Aebi's method [21]. Measurements were recorded at 240 nm at 15-second intervals for 1 minute using UV/visible light. CAT activity was expressed as μmol H_2O_2 consumed/min/mg protein.

2.6 Histopathological examination

Following the method described by Zemmouri et al. [22], lung tissues were collected and subsequently fixed in 10% formaldehyde. Afterwards, specimens underwent dehydration using ethanol solutions and then embedded in paraffin. Sections of 5 μm thickness were obtained from the paraffin blocks and stained using hematoxylin/eosin (H&E). The stained sections were examined using a LEICA DM 1000LED microscope.

2.7 Statistical analysis

All data were analysed using SPSS version 25. The *in vivo* data were expressed as mean ± standard error (SEM). All *in vitro* antioxidant tests have been expressed as an average of three replicates ± the standard deviation (SD). The IC_{50} and $A_{0.50}$ values were calculated by linear regression analysis. Group comparisons were performed by ANOVA one-way followed by the HSD Tukey post Hoc test. The level of statistical significance was determined at a p-value <0.05.

3. Results
3.1 Total bioactive content

The current study demonstrated the level of bioactive content in Cade oil as presented in Table 1. The total phenolic content in the Cade oil was assessed using The Folin–Ciocalteu method. Cade oil showed a high quantity

Table 1. Total polyphenols and flavonoids content of Cade oil.

Sample	Total phenolic (μg GAE/mg DW)	Total flavonoid (μg QE/mg DW)
Cade oil	275.21 ± 3.14	28.23 ±1.91

Data are expressed as mean ± SD of three parallel measurements. Total phenolic content is expressed as μg Gallic acid equivalents/mg of extract, and total flavonoids are expressed as μg Quercetin equivalents/mg of extract.

of phenolic content, reaching 275.21 ± 3.14 mg GAE/g DW. While flavonoids reached $28.23 \pm 1.91 \mu$g QE/mg, as indicated in Table 1.

3.2 *In vitro* antioxidant activity of Cade oil

To assess the antioxidant potential of Cade oil, various assays were employed, including DPPH and ABTS scavenging activity, β-carotene/linoleic acid bleaching capacity, Cupric reducing antioxidant capacity, Phenathroline and Galvinoxyl scavenging assays. The obtained results from these different tests were expressed in terms of IC_{50} and $A_{0.5}$ values, as presented in Table 2.

DPPH, a stable purple-coloured free radical, transforms to yellow hydrazine when reduced by an antioxidant. This characteristic was utilised in a DPPH assay to evaluate the radical-scavenging capability of Cade oil. The assay revealed that Cade oil possesses considerable antiradical capacity, reported by an IC_{50} value of 24.29 ± 0.39 μg/mL. However, this efficacy is lower compared to the well-known antioxidants BHA and BHT, which recorded IC_{50} values of 12.99 ± 0.41 μg/mL and 6.14 ± 0.41 μg/mL, respectively. Consistent findings were observed in both ABTS and Galvinoxyl Radical (GOR) assays, with Cade oil manifesting IC_{50} values of 24.44 ± 1.27 μg/mL and 21.92 ± 1.27 μg/mL, respectively. These results indicate a moderate level of antiradical efficacy relative to established antioxidants BHT and BHA.

The reducing ability of Cade oil was analysed using three distinct methods: reducing power, CUPRAC, and phenanthroline assays. The reducing power assay highlighted cade oil's potential in electron transfer, indicated by its effective conversion of Fe^{3+} to Fe^{2+} through potassium ferricyanide; exhibiting moderate activity with an $A_{0.5}$ value of 19.40 ± 0.45 μg/mL, surpassing that of BHT ($A_{0.5} > 50$ μg/mL). In the CUPRAC assay, based on the copper reduction method, Cade oil achieved a value of 30.40 ± 1.52, slightly lower than those recorded for BHT and BHA ($A_{0.5}$ values of 5.35 ± 0.71 μg/mL and 8.97 ± 3.94 μg/mL, respectively). The phenanthroline assay, assessing the reduction of metal iron, demonstrated Cade oil's effective potential, with an $A_{0.5}$ value of 11.52 ± 0.48 μg/mL, indicating a lower concentration requirement compared to BHT and BHA ($A_{0.5}$ values of 0.88 ± 0.04 μg/mL and 2.12 ± 0.04 μg/mL, respectively).

To conclude, the antioxidant capacity of Cade oil, the β-carotene bleaching assay, showed high activity with an IC_{50} value of 8.20 ± 0.25 μg/mL. However, this effectiveness was less than that of standard antioxidants BHT and

BHA, which displayed IC_{50} values of 1.11 ± 0.06 μg/mL and 0.88 ± 0.03 μg/mL, respectively.

3.3 *In vivo* antioxidant activity of Cade oil
3.3.1 Cell count levels in Bronchoalveolar BALF and serum

The findings regarding cell count in BALF and serum are depicted in (Fig.1), revealing notable results. In the BALF and serum of OVA-induced rats, inflammatory cells, including lymphocytes, monocytes and granulocytes, were evaluated. A significant cell number increment was noted ($p < 0.001$) in the OVA-challenged group compared to the control group. Importantly, administration of Cade oil significantly decreased ($p < 0.05$) the infiltration of these inflammatory cells both in BALF and serum relative to the OVA-challenged group.

3.3.2 Evaluation of the antioxidant defence status in lung and erythrocytes

In the lung and erythrocytes, Ova-sensitisation had a noticeable effect on the antioxidant defence level. MDA levels were significantly increased, while GSH, GPx and CAT levels were significantly decreased in the sensitised group compared to the control group (Fig. 2). Regarding the CO group, the treatment administration demonstrated a noteworthy improvement in all these parameters in the organs under study.

3.4 Histopathological results

The histological analysis assessed the pathological characteristics of OVA-induced allergic lung inflammation
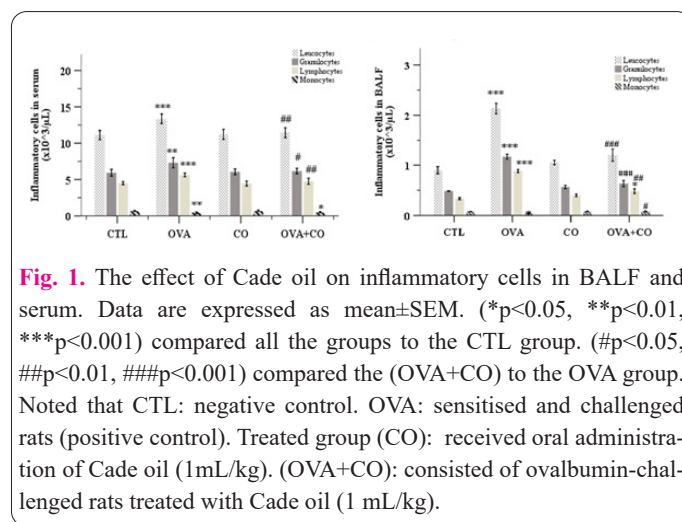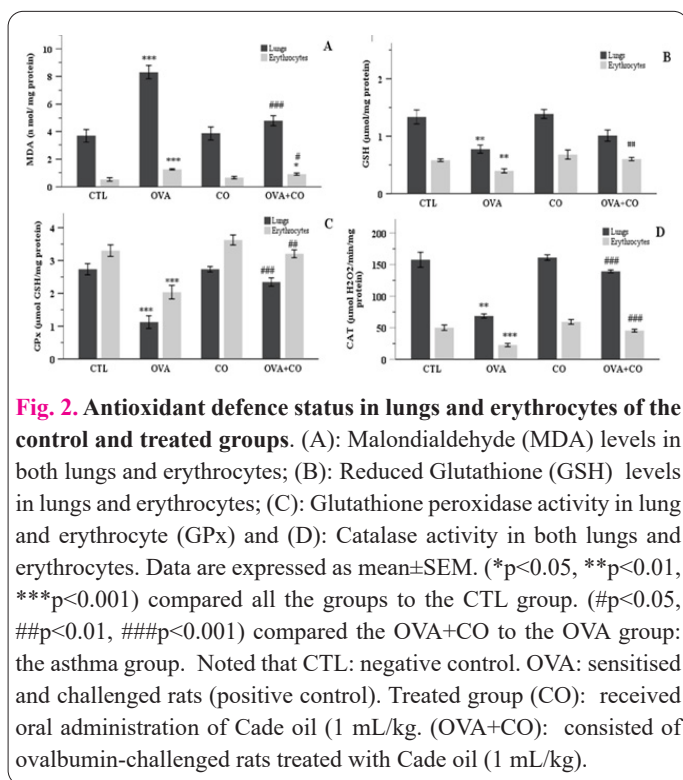

Fig. 1. The effect of Cade oil on inflammatory cells in BALF and serum. Data are expressed as mean±SEM. (*p<0.05, **p<0.01, ***p<0.001) compared all the groups to the CTL group. (#p<0.05, ##p<0.01, ###p<0.001) compared the (OVA+CO) to the OVA group. Noted that CTL: negative control. OVA: sensitised and challenged rats (positive control). Treated group (CO): received oral administration of Cade oil (1mL/kg). (OVA+CO): consisted of ovalbumin-challenged rats treated with Cade oil (1 mL/kg).

Table 2. Antioxidant activities of Cade oil.

	DPPH IC_{50}(μg/mL)	ABTS IC_{50}(μg/mL)	GOR IC_{50} (μg/mL)	β-Carotene IC_{50}(μg/mL)	Reducing Power Assay $A_{0,50}$ (μg/mL)	Cuprak $A_{0,50}$ (μg/mL)	Phenanthroline IC_{50}(μg/mL)
CO	24.29 ± 0.39^a	24.44 ± 1.27^a	21.92 ± 1.27^a	8.2 ± 0.25^a	19.4 ± 0.45^a	30.40 ± 1.52^a	11.51 ± 0.48^a
BHT*	6.07 ± 0.2^b	1.75 ± 0.2^b	3.32 ± 0.18^b	0.88 ± 0.03^b	$>50^c$	5.35 ± 0.71^b	0.88 ± 0.04^b
BHA*	12.9 ± 0.24^c	1.14 ± 0.11^b	5.38 ± 0.06^c	1.11 ± 0.06^b	NT	8.97 ± 3.94^c	2.12 ± 0.05^c
AA*	NT	NT	NT	NT	6.73 ± 1.15^b	NT	NT

IC50 and A0.50 values are expressed as the concentration of 50% inhibition percentages and the concentration at 0.50 absorbance, respectively. Both metrics, calculated by linear regression analysis and expressed as Mean ± SD (n=3). Different superscripts (a, b, c) in a column indicate significant differences, while identical superscripts (b, b) denote no significant difference, determined by ANOVA and Tukey's test (p<0.05). CO: Cade oil; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; AA: ascorbic acid; NT: not tested.

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Fig. 2. **Antioxidant defence status in lungs and erythrocytes of the control and treated groups**. (A): Malondialdehyde (MDA) levels in both lungs and erythrocytes; (B): Reduced Glutathione (GSH) levels in lungs and erythrocytes; (C): Glutathione peroxidase activity in lung and erythrocyte (GPx) and (D): Catalase activity in both lungs and erythrocytes. Data are expressed as mean±SEM. (*p<0.05, **p<0.01, ***p<0.001) compared all the groups to the CTL group. (#p<0.05, ##p<0.01, ###p<0.001) compared the OVA+CO to the OVA group: the asthma group. Noted that CTL: negative control. OVA: sensitised and challenged rats (positive control). Treated group (CO): received oral administration of Cade oil (1 mL/kg. (OVA+CO): consisted of ovalbumin-challenged rats treated with Cade oil (1 mL/kg).

(Fig. 3). Upon H&E staining, lung tissue of the OVA group (Fig. 3, B) exhibited a pronounced peribronchial inflammation by excessive leukocyte infiltration as compared to the normal tissue (Fig. 3, A). Additionally, there was an increase in the thickness of the respiratory bronchioles and an enhanced production of mucus by goblet cells. Interestingly, the (OVA+CO) group demonstrated enhanced lung morphology, characterised by reduced inflammatory cells, decreased thickening of airway cells and a decline in mucus production (Fig. 3, D). Moreover, the CO-treated group did not display any histological damage in the lung compared to the control group (Fig. 3, C).

4. Discussion

Oxidative stress is fundamentally implicated in the pathogenesis of a broad spectrum of chronic health conditions, including allergic asthma, highlighting the essential need to investigate antioxidant mechanisms for potential therapeutic strategies [5]. Despite extensive research in this area, the specific impact of natural remedies, such as Cade oil derived from *Juniperus oxycedrus*, on allergic asthma remain unexplored, with existing studies primarily limited to the extracts and essential oils derived from *Juniperus oxycedrus* [7, 23]. Addressing this gap, our investigation aims to elucidate the effects of Cade oil on allergic asthma, thereby contributing to a broader comprehension of its potential therapeutic benefits in oxidative stress-mediated conditions.

In response, a series of assays were conducted to ascertain the antioxidant potency of Cade oil. The significant antioxidant capacity found in our *in vitro* analyses prompted further investigations using a rat model of allergic asthma, which develops cellular and pathophysiological characteristics similar to those observed in human asthma [16], notably oxidative stress feature, a fundamental element implicated in the onset and progression of asthma [5].

Our results suggest that Cade oil possesses notable antioxidant activity, primarily due to its abundant phenolics and flavonoid content. Remarkably, the therapeutic properties of many bioactive compounds, especially herbal polyphenols and flavonoids, stem from to their redox potential, which plays a critical role in electron transfer processes, neutralization of free radicals, and chelation of metal ion [24].

In the context of asthma, inflammatory cell activation, particularly by T-helper 2 (Th2) cells, is crucial in in directing and managing the immune-inflammatory response in asthmatic airways. Following exposure to the ovalbumin allergen, the interaction between antigen-presenting cells and sensitised helper T lymphocytes result in increased secretion of several airway cytokines, notably IL-4, IL-5, and IL-13 [1].

These cytokines are fundamental in eosinophil differentiation, survival, and function, leading to the recruitment of inflammatory cells to the lungs, increased mucus secretion, enhanced airway hyperresponsiveness and allergen-specific IgE production [1, 5]. Moreover, inflammatory epithelial cells markedly produce pro-inflammatory cytokines and chemokines, such as such as tumour necrosis factor α (TNF-α) and interleukin-1 β (IL-1 β) [17].

TNF-α is involved in orchestrating the airway inflammatory, acting as a chemotactic agent for recruiting neutrophils and eosinophils, and enhancing the expression of adhesion molecules on the epithelial cell surface [25]. On the other side, IL-1β triggers the generation of Th2 cells following allergen exposition, leading to eosinophils activation and subsequent cytokines release, including IL-5 [25]. Animal models studies have consistently demonstrated a remarkable upsurge in TNF-α and IL-1β gene expression in OVA-induced asthma [5, 16, 25]. Arguably, reducing the inflammatory cell cytokines and chemokines in the airway using the anti-TNF-α and IL-1β drugs represent an efficient therapeutic strategy for treating asthma [26].
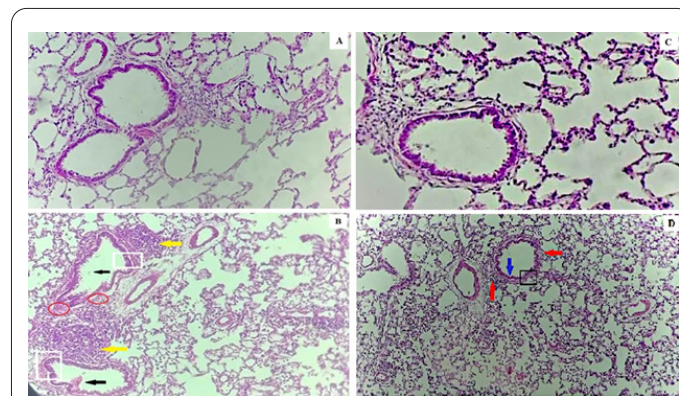

Fig. 3. **Histopathological analysis of the lungs section of rats.** A: Control group (x40). B: Asthma group (x40). C: Normal group treated only with Cade oil 1mL/kg /day orally (x40). D: Asthma group treated with Cade oil 1mL/kg /day orally (x40). The histological structure of the control rat exhibits a normal appearance. In contrast, the Ova-sensitised lung presents evidence of inflammatory cell infiltration in the bronchial airways (indicated by the yellow arrow), an increase in the thickening of a respiratory bronchiole (depicted by the white square), hyperplasia of goblet cells (shown by the red circle), and production of mucus (highlighted by the black arrow). Conversely, in the OVA+CO rat lung, a reduction in inflammatory cell presence (indicated by the red arrow), a decrease in the thickening of airway cells (represented by the black square), and a decrease in mucus production (illustrated by the blue arrow) are observed. The CO-treated rat lung exhibits a normal cellular appearance comparable to the control group.

Consistent with the findings of Chalchat et al. [27] and Barrero et al. [28], the major component present in Cade oil is δ-cadinene, known for its anti-inflammatory effects and ability to reduce IL-1β and TNF-α levels [29]. Our data indicates clearly that the oral administration of Cade oil reduced lung inflammation by decreasing leukocyte rates to their physiological ranges and minimising inflammatory cell infiltration into lung tissue, especially eosinophil and lymphocyte levels in OVA+CO group compared to the OVA group. These findings indicate that Cade oil had a therapeutic influence on the allergic response observed in Ova-sensitised rats, suggesting that δ-cadinene directly contributes to the oil's capacity to mitigate allergic responses in sensitized rats, aligning with previous findings that associate this compound with anti-inflammatory activity and a reduction in pro-inflammatory cytokines such as IL-1β and TNF-α. Therefore, may be attributed significantly to different phenolic compounds present in Cade oil, which not only neutralise free radicals but also demonstrate anti-inflammatory properties, contributing to the its inhibitory impact on allergic responses in sensitized rats.

Oxidative stress represents a fundamental hallmark of asthma, characterised by an imbalance between a heightened oxidant system and reduced antioxidant defences. The presence of high oxidant rates serves as an indicative marker of the ongoing inflammatory processes [5, 30]. In this context, numerous studies have investigated the bond between oxidative stress and asthma, focusing on a possible therapeutic strategy revolves around antioxidants [31, 32].

Particularly, oxygen-derived free radicals, especially frompolyunsaturated fatty acids, lead to lipid peroxidation within the cell membranes, producing Malondialdehyde (MDA), known as an indicator of oxidative stress due to its oxidant properties [33]. Hence, the accumulation of MDA in lung tissue could potentially enhance the leukocyte chemotaxis, releasing an excess of ROS production by immune cells infiltrating airway inflammation, leading to asthma-related airway injury [33].The present investigation showed that the OVA challenge significantly increased MDA amounts in lungs and erythrocytes samples; this aligns with the research of Khaldi et al. [31], who investigated the effects of *Nigella sativa* oil on lungs inflammation and oxidative stress. Interestingly, the administration of Cade oil significantly reduced the oxidative alteration of lipid peroxidation levels in the OVA+CO group, illustrating its potent antioxidant properties.

The human body has several mechanisms that ensure its protection against free radicals, including endogenous non-enzymatic antioxidants such as GSH and enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) [34, 35]. These systems help in neutralising free radicals and protecting biological targets from oxidative harm, thereby aiding in disease prevention [34]. Our data demonstrated that rats exposed to OVA showed clear signs of oxidative stress, as indicated by reduced amounts of antioxidant enzymes GSH, GPx and Catalase, accompanied by an increase in MDA levels. Accordingly, it may be suggested that oxidative stress provoked increased inflammation, given the significant presence of inflammatory cells in the OVA-induced asthmatic rats [22, 35].

GSH, a vital antioxidant, plays a crucial role in cellular defense against oxidative injury, especially in the lungs [22, 30]. As a non-protein thiol-containing compound, GSH serves as a co-substrate for glutathione transferase (GST) and GPx, reducing oxidative damage caused by reactive oxygen species [30]. Additionally, in cells, glutathione interacts with free radicals to generate glutathione in its oxidised form (GSSG). The latter is efficiently regenerated into its reduced state (GSH) by GSH reductase, using NAD(P)H as the electron donor [2].

In this research, the OVA challenge significantly decreased glutathione (GSH) levels, indicating impairment in the antioxidant defence system and potentially leaving the lung tissues vulnerable to oxidative damage and inflammation. These results are consistent with earlier studies [22, 31, 32]. which also reported a similar decline in GSH levels in the context of allergic lung inflammation. Furthermore, these results revealed that GSH values were elevated in the treated group, indicating the antioxidant properties of Cade oil that can protect against oxidative stress through its effective role as a scavenger of free radicals.

Additionally, endogenous antioxidant enzymes, such as GPx and CAT, have been evaluated in order to determine the extent of oxidative stress. A notable decrease in catalase and GPx activity was noticed in the lungs of the Ova group when compared to the Control group, indicating deterioration in the antioxidant function, pointing to elevated oxidative stress. Catalase has been regarded as a critical antioxidant enzyme in the lungs, responsible for neutralising reactive H_2O_2 into H_2O and O_2 [34]. The depletion of CAT activity can be attributed to its inactivation caused by elevated synthesis and accumulation of hydrogen peroxide (H_2O_2) and superoxide radicals within the asthmatic lungs [22]. Moreover, GPx is an intracellular, selenium-containing enzyme known to catalyse the reduction of H_2O_2 to water [33], reinforces the importance of GSH as a necessary cofactor [2, 34].

The current histological investigation of the lung is consistent with earlier findings; as illustrated in (Fig. 3), the control group demonstrated normal lung histology, with no observed inflammatory cell infiltration or airway remodelling, reinforcing that the Control group is a suitable comparison group. On the other hand, the Ova group showed a massive inflammatory cell infiltration involving the recruitment and activation of various immune cells, including eosinophils, lymphocytes, mastocytes, and macrophages. These inflammatory cells release cytokines, chemokines, and other mediators that promote bronchial epithelium damage and increase cell proliferation, leading to the thickening of the bronchiolar walls [22]. The presence of inflammatory mediators can also stimulate goblet cells to undergo hyperplasia and produce excessive mucus within the lumen of the bronchioles, which can further contribute to airway obstruction and thickening [17]. Interestingly, lung morphology significantly improved in the OVA+CO group after receiving treatment with Cade oil. The reduction in inflammatory cell infiltration, airway thickening, and the decline in mucus production indicate a potential protective effect of Cade oil treatment against allergic lung inflammation.

5. Conclusion

This study explored the antioxidant, anti-inflammatory and anti-asthmatic properties of Cade oil through both in

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vitro and in vivo experiments. Our research yielded seve-ral significant insights. Firstly, Algerian Cade oil is rich in phenolic compounds, which could explain its impres-sive antioxidant and anti-inflammatory activities observed in vitro. Additionally, the administration of the Cade oil in an OVA-induced asthma model has counteracted oxi-dative stress by neutralising ROS, minimising pulmonary inflammation through modulating immune cell infiltration, as well as protecting membrane lipids from peroxidation, enhancing the activity of antioxidant enzymes, besides improving lung morphology by reducing the alteration of the airways. Future research involving in vitro and in vivo toxicological assessments will be necessary to establish the safety of this oil for human consumption. It is crucial not only to confirm its safety but also to identify and iso-late the active compounds that hold potential therapeutic value, which might serve as promising components for novel pharmaceutical formulations.

Conflict of interest
The authors report no conflicts of interest related to this article.

Consent for publications
The authors read and approved the final manuscript for publication.

Ethics approval and consent to participate
The methodologies applied in this study received appro-val from the Ethical Committee of the Directorate General for Scientific Research and Technological Development, which operates under the auspices of the Algerian Mi-nistry of Higher Education and Scientific Research. This endorsement was formalised with the issuance of license number PNR/SF 08/2012, ensuring adherence to ethical standards in the research process. Rigorous measures were employed to reduce to a minimum the number of animals used and mitigate any distress they might experience.

Informed consent
The authors declare not used any patients in this research.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request

Author's contributions
MA: Conducted all laboratory procedures and prepared the manuscript. MA, M HZ, ND, TK: Conducted animal experiments, ensuring adherence to ethical guidelines and proper data collection. CB: Supervised experiments and provided laboratory resources at CRBT. MA and LK: Conducted statistical analysis and data interpretation, HA: Conducted the microscopic analysis for the histolo-gical study. MZ: methodology, MM: provided laboratory resources at LBTE. AB and MB:Designed the research framework, supervised the overall project to ensure cohe-rence and scientific integrity. All authors: Contributed to manuscript revision, provided critical feedback, and ap-proved the final version for publication.

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