



Evaluation of CNS Activity and Anti-Inflammatory Effect of *Pistacia atlantica* Desf. Essential Oil from Morocco

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Abstract This work was carried out within the framework of the valorization of the Moroccan aromatic and medicinal plants, we chose to study *Pistacia atlantica* Desf. whose use for a long time by the populations, in the nutrition and the traditional medicine.

The objective of this study is to determine the yield of *Pistacia atlantica* Desf. fruit essential oil (PAEO) extracted by the hydrodistillation method and to investigate the chemical composition by gas chromatography-mass spectrometry (GC/MS). The central nervous system (CNS) activity of PAEO was investigated using different models based on mice and rats behavior and anti-inflammatory effect were performed using the carrageenan induced hind paw edema test.

Pistacia atlantica Desf provided a 0.058% yield of essential oil with constituents representing approximately 97.35% of the total oil, the major compounds are: anisole (25.62%), cineol (1.4) (16.88%), sabinene (14.45%) and cresylacetate para (8.55%).

In the pharmacological activities tests it was found that the pretreatment of *Pistacia atlantica* Desf. fruit essential oil has a potent sedative and anti-inflammatory properties.

Keywords *Pistacia atlantica* Desf, essential oil, yield, GC/MS, psychotropic, anti-inflammatory

Introduction

Many plants have been reported to use in the aromatherapy due to presence of essential or volatile oils in different plants materials like flowers, barks, stem, leaves, roots, fruits *etc.*

The therapy of essential oils from medicinal plants is known to relieve the stress, rejuvenate and regenerate the individual for a next day's work. Olfactory nerves from nose to the brain are the site of action for these essential oils. These oils have well proven antibacterial, antibiotic, and antiviral properties and many published reports elsewhere as well as folkloric practitioners have suggested them to be useful in many other diseases like Alzheimer, cardiovascular, cancer and labor pain in pregnancy *et c*[1]. There is an increased trend nowadays to use this therapy in the treatment of cancer and sleep disorder [1]. Their organic character and to act in a supportive manner with the body, provide a feeling of well-beingness [1].



In Morocco the traditional uses of plants for medicine were studied. To this date, 231 medicinal plants belonging commonly to the Moroccan pharmacopoeia have been identified and are presented in a table with the vernacular name in Arabic and/or Berber, the ecological distribution, the useful part and the medicinal use. The study of the main therapeutic indications of the medicinal plants gives a clear picture of the health problems which are treated by traditional medicine in Morocco.

The genus *Pistacia* has numerous species and their essential oil composition have been studied by several authors [2-3]. *Pistacia atlantica* Desf. (PA) is an indigenous medicinal plant which has been used as a traditional medicine for the treatment of various ailments for over a century. *Pistacia atlantica* Desf. is an aromatic member of the Anacardiaceae family.

Our work is aimed to study the chemical composition and the psychopharmacological analysis behavior and the anti-inflammatory effect in rodents of *Pistacia atlantica* Desf. which is the object of our concern.

Experimental

Materials and Methods

Plant Material

The plant material (fruits) growing spontaneously on the East of Morocco (Oujda) was collected during 2015. A voucher specimen (101537) was deposited in the Herbarium of Botany Department of Scientific Institute of Rabat [4].

Extraction of the Essential Oil

The fresh fruits were washed under running tap water to remove adhere dirt, followed by rinsing with distilled water, shade dried and each 549 g of fresh fruits were hydro-distilled using a Clevenger apparatus for 3 h. The extraction was repeated two times and the obtained oils were pooled separately, dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4 °C in amber glass vials until analysis.

Identification of the Essential Oil Composition

Gas Chromatography /Mass Spectrometry (GC/MS) Analysis

GC-MS analysis of the EO was performed on a PERKIN ELMERXL equipped with capillary column PERKIN ELMER ELITE (60m x 0.32mm x 1.00 μm film thickness). The oven temperature was programmed from 50°C to 230°C at 4°C/min.

Nitrogen was the carrier gas at 0.8mL/min flow rate. The components of the oil were identified by comparison of their mass spectra with those in the Wiley-NIST 7th edition library of mass spectral data. The percentage composition of the oil sample was calculated from GC-MS peak areas [5].

Pharmacological Evaluations

Animals

Normal healthy male Wistar rats weighing 200-250g and Swiss Mice weighing 20-30g were used in the pharmacological tests and females Swiss mice in the LD50 calculation test.

They were housed in standard conditions (approximately 26°C, 60–70% relative humidity and 12h-dark/12h-light cycle). The animals received a standard rodent diet and water *ad libitum* except when fasting was required in the course of the study. The animals were obtained from the animal centre of Mohammed V University, Medicine and Pharmacy Faculty, Rabat, Morocco. The experiments were performed following the guidelines set for the international association for the study of pain and the national institute of health (NIH publication N°. 85-23, 1985) regarding the care and treatment of experimental animals [6,7].

Acute Toxicity test

Acute toxicity study was carried out using the OECD (Organisation for Economic Co-operation and Development Guidelines 423).

Healthy female Swiss mice (generally slightly more sensitive) fasted 3 h before the experiments with water *ad libitum*. Animals were randomly divided into three groups (n = 3). The first group (control group) received orally



peanut oil (vehicle control). The second and third groups were treated with *P. atlantica* Desf. essential oil at the doses of 2 and 5 g/kg respectively.

Animals were observed for their general behavioral symptoms, body weight changes, hazardous symptoms and mortality during the first 6 h and subsequently for the next two weeks after administration of *P. atlantica* Desf. essential oil. The lethal dose of 50% (LD_{50}) was estimated according to the method described by OECD Guidelines 423[8].

Psychopharmacological Screening

Hole-Board Test

The board is 40cm x 40cm and 2.2cm thick. It has 16 holes of 3cm diameter and it is made of grey Perpex. The matt finishing of the upper panel avoids reflections which may alter the animal behavior. The mice were injected with drugs or vehicle and, thirty minutes later, each animal was placed in the center of the hole-board, and allowed to freely explore the apparatus for 5 min. The numbers of head pokes and the time of dipping during a 5 min period were recorded [9].

Muscle Relaxant Activity

Rotarod Test

Untreated mice were placed on the revolving bar of the Rota-Rod apparatus (Ugo Basile, Model 7600) for two consecutive periods of 60s at a speed of 12 rpm. Animals were divided in to 4 groups of 5 animals each. The first two groups were administered with peanut oil and Bromazépam (20mg/kg). The other 2 group were administered with different doses of PAEO (300 and 500 mg/kg). Then the animals were placed on the rod. The time taken for the mice to fall from the rotating rod was noted. Motor performance was evaluated at 30, 60, and 120 min following treatments, and the amount of time of permanence(s) on the revolving bar during a 60 second period was recorded [10].

Traction test

Forepaw of a mouse was placed on a small twisted wire rigidly supported above with a bench top. Normal mice grasped the wire with forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 sec. Inability to put up at least one hind foot constituted fail to traction. The test was performed in animals after injection of essential oil of *P. atlantica* Desf. at doses of (300 and 500 mg/kg) and Bromazépam(20 mg/kg) and the response were recorded[11, 12].

Chimney Test

Chimney test of Boissier 1961 was used where each mouse was introduced into the vertical glass tube 30cm in length and 28mm diameter, with the head forward. When the mouse reached the other end of the tube, the tube was moved to a vertical. The mouse tried to climb backwards.

The time required for the mouse to climb backwards out of the cylinder was noted. Cut off time was 240 sec. A normal mouse typically attempts to escape in thirty seconds, and the mice considered as subject to the sedative effect when performing the rise of cylinder greater than 30sec [13, 14].

Light/dark Test

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light. The apparatus consisted of two 20 cm X 10 cm X 14 cm plastic boxes: one light compartment painted white and brightly illuminated and the other was dark painted black and dimly illuminated with red light. The mice were allowed to move from one box to the other through an open door between the two boxes. The illumination in the black compartment was 50 lux, in the white area it was increased to 1000 lux, generated by an extra light source. A mouse was put into the light box facing the hole. The transition between the light and the dark box and time spent in the light box were recorded for 5 min. Every time before placing each animal, the maze was cleaned with 5% alcohol to eliminate the possible bias due the odor left by the previous animal [15].



Hypnotic test

The animal is considered as in state of hypnosis, when placed on his back, it loses the righting reflex. Following the oral administration of *P. atlantica* essential oil at the dose 700mg/kg the time between loss and recovery time of righting reflex was recorded as sleeping time [16, 17].

Catalepsy test

Catalepsy test consists of placing an animal into an unusual posture and recording the time taken to correct this posture.

The rat is considered as in state of cataleptic when they agree to pass the forelegs with the hind legs ipsilateral. After administration of dose (700mg/kg) of the essential oil, 5 rats are labeled differently so as to monitor the evolution of catalepsy individually over time. Every 15 minutes we follow the evolution of catalepsy. For each rat the onset time of this catalepsy was recorded [18].

Thiopental sodium Induced sleeping time

Hypnotic effect method based on potentiation of thiopental induced sleeping time by essential oil of *P. atlantica* was used to study the effect of plant materials. A sub-hypnotic dose of thiopental (40mg/kg) was injected via intraperitoneal route, 30min after a similar injection of vehicle or the drug. The standard drug used was Bromazepam (30mg/kg P.O.). The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance and reappearance of the righting reflex [19].

In Vivo Anti-Inflammatory Activity

The evaluation of the anti-inflammatory activity of *P. atlantica* Desf. essential oil was carried out by using the chemical stimuli (Winter Test) [20] induced paw edema in rats. In this method, all animals were fasted 18 h before testing and received 5 mL of distilled water by gavages to minimize individual variations in response to the swelling of the paws. The right hind paw (RP) is not treated, and it is taken as control.

Carrageenan-Induced Rat Paw Edema

The carrageenan-induced paw edema model [21] was used to evaluate the anti-inflammatory effect of *P. atlantica* Desf. essential oil. The initial paw volume was recorded using an Ugo Basile model LE750 plethysmometer.

Rats groups were orally administered essential oil of *P. atlantica* Desf. (100 and 200 mg/kg); Indomethacin (10 mg/kg) was used as reference drug while distilled water (5 mL/kg) was used as negative control. After 60 min had passed, carrageenan (0.05 mL of a 1% w/v solution, prepared in sterile saline) was injected subcutaneously into subplantar region of the left hind paw of each rat. The right hind paw is not treated; it is taken as a witness. One hour 30 minutes, 3 hour and 6 hours after the injection of carrageenan, the paw volumes of each rat were measured. Mean differences of treated groups were compared with the mean differences of the control group. The percentages of inhibition of inflammation were calculated according to the following formula:

$$\% \text{ of inhibition} = \frac{\text{mean } [V_{\text{left}} - V_{\text{right}}]_{\text{control}} - [V_{\text{left}} - V_{\text{right}}]_{\text{treated}}}{[V_{\text{left}} - V_{\text{right}}]_{\text{control}}} * 100,$$

where V_{left} is the mean volume of edema on the left hind paw and V_{right} is the mean volume of edema on the right hind paw.

Statistical Analysis

All Results were expressed as Mean \pm SEM. The significance of difference between means was determined by student's t-test, followed by One-way ANOVA. A level of significance ($P < 0.001$) was considered for each test.



Results and Discussion

Natural products and herbal medicines are a promising source of new therapeutic agents and for the development of complementary and alternative medicines to conventional drug regimens [22]. These medicinal plants were employed to prevent stress, ageing, increase longevity, anxiety and offer resistance to diseases by augmenting the immune system.

In our study the mean yield of PAEO obtained from fruit was 0.058%. In our study the oil yield of *P. atlantica* Desf. seems to depend on the nature of parts of plants used for extraction and also on the mode of extraction. The GC-MS analysis of the essential oil, obtained from steam distillation of *P. atlantica* has led to the identification and quantification about 16 components, comprising over 97.35% of the essential oil.

The results obtained (**Table 1**) by GC-MS show that the main constituents of the oil were: Anisole (25.62%), Cineol (1.4) (16.88%), Sabinene (14.45%), Cresylacetate para (8.55%), cresol (meta) (2.07%), Terpeneol alpha (1.47%) and Calamenene «1S, trans» (1.16%). The results of this study show both qualitative and quantitative differences with oils from *P. atlantica* Desf. of other countries.

It seems that environmental factors such as geography, temperature, day length, nutrients, etc., were considered to play an important role in the chemical composition of *P. atlantica* Desf. essential oil of Morocco.

Table 1: The chemical constituents of the essential oil of *Pistacia atlantica* Desf. obtained by GC-MS

TR	%	Composés
14.945	25.62	Anisole
19.295	16.88	Cineol (1.4)
17.422	14.45	Sabinene
25.779	8.55	Cresylacetate para
16.88	4.82	Camphene
18.992	3.79	Hexylacetate (n)
20.515	3.77	Ocimene (trans)
35.195	3.59	Nerylacetone
26.245	3.15	Terpeneol 4
15.568	3.12	Thujene (alpha)
18.083	2.69	Octanol (3)
18.635	2.22	Carene (2)
21.825	2.07	Cresol (meta)
29.938	1.47	Terpeneol alpha
38.501	1.16	Calamenene « 1S, trans »
Total		97.35

In one hand in the acute toxicity test, our essential oil did not reveal any signs of toxicity or mortality in any animal, during the 14 days observation period so the LD₅₀ of PAEO was estimated to be greater than 5000 mg/kg. In the other hand, the effect of PAEO fruits were studied in several behavioural animals model for the evaluation of central activity, and providing information about myorelaxant, anxiety and depressant activities. The results of PAEO psychotropic effects were expressed by comparison with control and reference groups (**Table 2**).

In the **traction test** the mice treated with the essential oil of *Pistacia atlantica* Desf. showed a significant failure in traction at all doses tested with a high percentage of 60 % at the dose 500 mg/kg when compared with the control group. Besides in the **chimney Test** the rodents treated with the essential oil of *Pistacia atlantica* DESF. at doses of 300, and 500mg/kg, do show loss of initiative and curiosity ($P < 0.001$) (**Table 2**).

Also in the **hole board Test** the PAEO at doses (400 and 500mg/kg, p.o.) significantly reduced head-dip (both $P < 0.001$) when compared with vehicle alone (**Table 2**). And in the **Rota Rod Test** the effect of this essential oil on motor coordination at doses (300 and 500mg/kg) showed a significant effect on the spontaneous motor activity in mice. The standard drug (Bromazepam) also showed highly significant effect when compared to control ($P < 0.001$).



The results of the **Hypnotic test** demonstrated that PAEO at dose of 700mg/kg have no hypnotic effect on the animals during this experiment **also** the result of the **Catalepsy test** indicated that PAEO did not induce catalepsy in rats.

Table 2: Sedative effect of the essential oil of *Pistacia atlantica* Desf. in mice

Test		Control	Bromazépam 20mg/kg	EOPA 300 mg/kg	EOPA 500 mg/kg
Traction Test	Percentage of falls (%)	0	100	40	60
	Re-establishment Time	0.1 s. ± 0.0	10 s.± 0.3*	19.83 s.±18.12	21.99s- ±14.98
Chimney Test	Percentage of mice that responded to the test (%)	0	100	40	60
	Time to go back the tube in seconds	6 s. ± 0.5	> 2 min*	39.81s. ± 18.49	49.23s. ±31.55
Hole-board Test	Explored holes during 5 minutes	7 ± 1	0 ± 0.0*	0.28 ± 0.11 n=5	0.72 ± 0.75
Rota rod Test	Time of permanence (s)/60s	120 s. ± 0	1.2s. ± 0.90	25.06s. ± 21.46	16.06 s. ± 8.89

Values are mean ± S.E.M (n = 5); p.o. means oral route; n means number of mice per group; sec means seconds; EOPA means the essential oil of *Pistacia atlantica* Desf; P < 0.001 versus the control group.

In the potentiation of Thiopental sleep test, PAEO did not increase the sleeping time of the rats at the dose of 40mg/kg compared to control (**Table 3**).

Table 3: The effects of the essential oil of *Pistacia atlantica* Desf. on Thiopental sodium Induced sleeping time

	Control Thiopental sodium 40 mg/kg	Reference Thiopental sodium 40 mg/kg + Bromazepam 50mg/kg	Thiopental sodium 40 mg/kg + 40mg/kg EOPA
Sleep latency (min)	4 ± 1 min	1 min 48 ± 3.16	0 ± 0
Sleeping time (min)	25 ± 0.5 min	2h0 min 30s ± 2.73	0 ± 0

EOPA means the essential oil of *Pistacia atlantica* Desf. Data are expressed as mean ± S.E.M (n = 5); P < 0.001 versus the control group.

Anxiety is a negative emotion that occurs in response to perceived threats can come from internal or external factors and can be real or imaginary. The incident of the anxiety in the community is very high and associated with many diseases and morbidity.

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic, serotonergic neurotransmission in etiology, expression and treatment of anxiety. The adrenergic and dopaminergic systems have also been shown to play a role in anxiety [13].

In the current work we examined the anxiolytic effects of PAEO using the Light/dark exploration test (**Table 4**). This test is an ethological based approach avoidance conflict test and it is sensitive to drugs that affect the anxiety. [12]

Mice tend to explore a novel environment, but to retreat from the aversive properties of a brightly-lit open field. In a two-chambered system where the animals can freely move between a brightly-lit open field and a dark corner, they show more increasing time spending action in light chamber due to anxiolytic agents.

Our results showed that the EOPA (300 mg/kg) increased time spent in the light chamber, suggesting anxiolytic action.



Table 4: The effects of the essential oil of *Pistacia atlantica* Desf. on Light/dark test

Treatment	Dose (mg/kg)	Time in the light box	Time in the dark box	No. of transition
Control	-	310.06 ± 15.44	169.31 ± 11.22	11 ± 1.8
Diazepam	3	392.5 ± 16.00	108.5 ± 9.1	14.25 ± 2.28
EOPA	300	212.60 ± 156.59	87.52 ± 50.23	43 ± 4.97

Values are mean ± S.E.M (n = 5); EOPA means the essential oil of *Pistacia atlantica* Desf.; *P < 0.001 versus the control group.

Figure 1 illustrated the anti-inflammation effects of essential oil of *P. Atlantica* on carrageenan-induced inflammation: measured 1h30, 3h, and 6h after injection of the phlogistic agent. All doses of the essential oil showed significant ($P < 0.001$) anti-inflammatory effects from 1h30 to 6h with a percentage of 85.29%, 69.23% and 85.29% respectively at the dose 100mg/kg. Results obtained with essential oils were better than those obtained with indometacin during the experimental period.

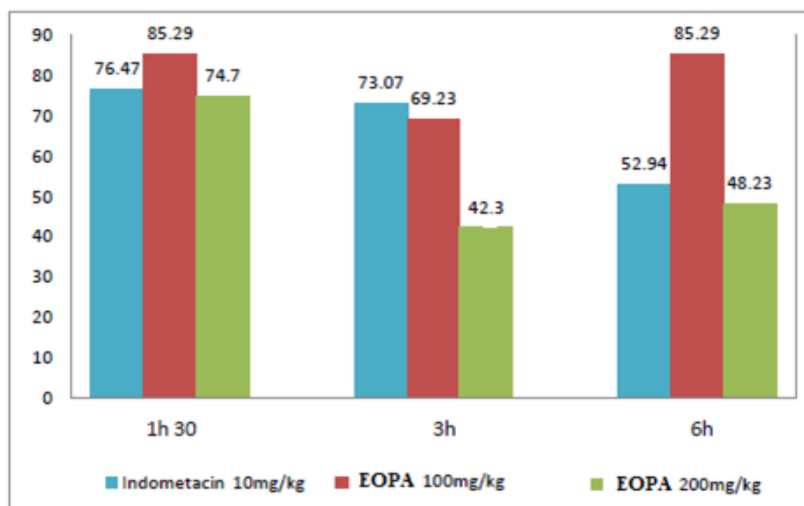


Figure 1: Percentage of inhibition of inflammation of essential oil of *Pistacia atlantica* Desf. using carrageenan-induced rat paw edema

Table 5: Effect of essential oil of *Pistacia atlantica* Desf. On carrageenan-induced rat paw edema

Treatment groups n=6	Dose mg/kg p.o.	Mean volume of edema (paw left-paw right) induced by carrageenan (ml)		
		1h30	3h	6h
Control	-	0.17±0.013	0.26±0.001	0.34±0.023
Indomethacin	10	0.04±0.004*	0.07±0.005*	0.16±0.008*
EOPA	100	0.025±0.02*	0.08±0.08*	0.05±0.03*
EOPA	200	0.043±0.032*	0.15±0.07*	0.1±0.085*

N= 6; these results compared with standard drug (Indomethacin, 10mg/kg, p.o.) were administered by the oral route

Conclusion

The observation suggested that PAEO possess potent CNS depressant, anxiolytic effect and anti-inflammatory effect that might be associated with the major components and the minor components found in the essential oils.

Conflicts of interest

All contributing authors declare no conflicts of interest.



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