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In vivo analgesic and anti-inflammatory properties of the aqueous extract of *Pistacia atlantica* Desf. from Morocco

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ABSTRACT

The present study analyses the pharmacological activity *in vivo* models of the aqueous extract obtained from *Pistacia atlantica* Desf of Morocco. The plant selected for this study have been used in traditional medicine in Morocco for the treatment of various diseases that are considered as inflammation in nature, e.g. arthritis, rheumatism, fever, and related inflammatory diseases. The result of this study showed that the aqueous extract of *Pistacia atlantica* Desf. lacked toxicity, but exhibited a high analgesic effect in writhing Test and in tail immersion Test suggesting the induction of a peripheral and central analgesic response. The aqueousextract of this plant also exhibited an anti-inflammatory action inhibiting the rat paw edema induced by carrageenin and experimental trauma. We can conclude that the aqueousextractof *Pistacia atlantica* Desf. Possesses potential anti-inflammatory activities, supporting the traditional application of this plant in treating various diseases associated with inflammation and pain in Morocco.

Keywords: Anti-inflammatory, *Pistacia atlantica* Desf, Aqueous extract, Acute toxicity, carrageenin.

INTRODUCTION

In developing countries, it is estimated that about 80 % of the population really depends on traditional medicine for their primary healthcare. There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. In recent times,focus on plant research has increased and non-steroidalanti-inflammatory drugs constitute one of the most widely used classes of drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. Herbal medicines are in line with nature,with less hazardous reactions [1]. The resiniferous pistachio tree belongs to Pistacia, a genus of eleven species in the Anacardiaceae family distributed in the Mediterranean area [2]. The pistachio Atlas pistachiois the only tree of North-African steppe and is an important forestry heritage of the Eastern Region of Morocco. In Morocco, the *Pistacia atlantica* (Betoum) is a tree which can reach 20 m in height. It is a long-living, dioecious, deciduous, variable and is also named "BETM" [3]. In Morocco*, P. atlantica* is one of the plants widely recommended by herbalists because it is the source of mastic gum, an exudate which strengthens gums, deodorizes breath and combats coughs, chills and stomach diseases [4]. The fruits of *P. atlantica* are also used for tanning and as fodder for cattle. They contain oil, which is used for soap making. From the bark of the wood, a resin is collected for laquer production and it is also used in popular medicine (as an antiseptic to wounds, etc.) [5]. A reported hypoglycemic activity is probably in relation with its ability to inhibit the α-amylase activity [6]. On an ethnobotanical point of view, the oil from the fruit is used as an antidiarrheal [7]. This oil has good nutritive quality because of its content in unsaturated fatty acids (oleic + linoleic = 73%) and saturated fatty acids (palmitic + stearic = 25.8%) [8].

In spite of its wide traditional use, particulary in analgesic and inflammatory the plant has not yet been systematically screened in Morocco. Therefore, it was considered worthwhile to test the anti-inflammatory and analgesic activities of the *Pistacia atlantic*a Desf.

MATERIALS AND METHODS

Plant Material

Fresh leaves, unripe fruits and leaf-buds of *Pistacia atlantica* Desf. plants, growing spontaneously on the East of Morocco (Oujda) were collected during 2015. Voucher specimens are deposited in the Herbarium of the scientific insitute in Rabat [9].

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Preparation of the Aqueous Extract

Aerial parts of*Pistacia atlantica* Desf. were air-dried and ground into a fine powder. 50g ofpowder was macerated for 24 hrs in 500ml of distilledwater. A percentage yield of15.3% was obtained after extraction and concentration underreduced pressure on a rotary evaporator attached to a vacuum pump and stored at atemperature of 4°C until use [10, 11].

Animals

Experiments were performed on 180-220g wistar rats and on 25-30g adult mice in their 8-9 weeks, the rodents were obtained from the animal center of Mohammed V University, Medicine and Pharmacy Faculty, Rabat, Morocco. Animals were housed 6 per cage in temperature and humidity controlled environment under a 12h light/ dark cycle. Food and water were available *adlibithum*. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991 and approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509. All efforts were made to minimize the number of animals which were used and their suffering degree [13, 14].

Acute toxicity test

Female albino mice (n = 3 per group) were treated with different doses of the extracts (300, 2000 mg/kg, p.o.)as described by OECD 423. The number of deaths was counted at 48 h after treatment; the rodents were kept under observation for a period of 14 days [15].

Antinociceptive activity

Central and Peripheral analgesic activities of the tested extract was carried out in rodents by using acetic acid-induced writhing and tail immersion methods, respectively.

Acetic acid-induced writhing response in mice

The abdominal constriction test described by Koster *et al*. (1959) was used. The mice were divided into groups of six. 30 minutes after the administration of the extract, the mice were given an intraperitoneal injection of acetic acid solution(3% with 300 mg/kg). The number of writhes produced in these animals was counted for 20 min. The aqueous extract of PA was administered in different doses at (200, 400 and 600mg/kg, p.o.) to the Swiss miceafter an overnight fast [16].

Tail immersion test

The tail immersion test was assessed on rats using thermal stimuli method [17, 18]. Briefly, rats in groups of 6 were placed in individual restraining cages and the nociceptive reaction time, in set, was determined when the tail was immersed in a constant-temperature water bath maintained at 55°C. The cut-off time was 10 Sec imposed for all animals that failed to respond to the stimulus. The aqueous extract of PA at 200,400 and 600mg/kg were given orally by intubation. The initial reading started immediately before administration of test and standard drugs (Morphine 5 mg/kg s.c) and then 15, 30, 45, 60 and 120 minutes after the administration.

***In Vivo* Anti-Inflammatory Activity**

Carrageenan-Induced Rat Paw Edema

Male and female Wistar rats (180–220 g) were treated withaqueous extractof *Pistacia atlantica* Desf. (AEPA) at a dose of 200and 400 mg/kg or vehicle. One hour after administration of the extracts, the rats were injected into the plantar aponeurosis of the left hind paw with carrageenan (0.05 ml, 1%). Edema was measured plethysmometer Digitals 7500, 1h30, 3 and 6 h later. The difference in the left paw and right paw volumes indicated the volume of inflammation [19].

% of inhibition=

mean [v Left _v Right] control – [v Left _v Right] treated ⁄ [v Left _v Right] control × 100.

V Left means volume of edema on the left hind paw and **v Right** mean volume of edema on the right hind paw.

Experimental Trauma-Induced Rat Paw Edema

Paw edema model: On the morning of experiment, rats were weighed and baseline paw volume was measured with the aid of plethysmometer Digitals 7500. To ensure uniformity, lateral malleolus of left hind limb was marked in all animals so that the same length of paw is dipped in fluid each time. This was followed by administration of drugs. After 60 min of drugs administration,

A weight of 50 g was made to fall onto the dorsum of the left hind-paw of all animals for inducing inflammation. Paw volume was again measured after 1h30, 3h and 6 h of sub-planter injection of 1 percent carrageenan. The paw volume and percent decrease in paw edema was compared between control group and drug-treated groups [20].

% of inhibition=

mean [v Left _v Right] control – [v Left _v Right] treated ⁄ [v Left _v Right] control × 100.

V Left means volume of edema on the left hind paw and **v Right** mean volume of edemaon the right hind paw.

Statistical analysis

The results were expressed as mean ± S.E.M. statistical analysis of data was done using one way analysis of variance followed by student's test. A p value less than 0.05 were considered significant.

RESULTS AND DISCUSSION

In folk medicine, plants play a very important role in human life since the ancient time, not only as a source of food, but also in treatment of various diseases. Medicinal herbs have been used as a form of therapy for the relief of pain throughout history [21] in addition and there has been a growing interest in plants as a significant source of new pharmaceuticals [22]. The results of the present study revealed the antinociceptive and anti-inflammatory effects of *Pistacia atlantica* Desf. aqueous extract in animalmodels.

In the acute toxicity test aqueous extract of PAdid not produce any behavioral changes and mortality in mice in doses up to 2000 mg/kg. Accordingly, it suggested that oral LD_{50} of the tested extract was higher than 2g/kg. Therefore, the tested plants can be categorized as safe [23]. Acetic acid induced writhing and tail immersion test are models of pain that mainly involve peripheral and central mechanisms respectively [24]. For the acetic acid induced writhingsignificant protection against writhing was observed in animals treated with AEPA (Table 1).All doses of AEPA (200, 400 and 600 mg/kg)induced an analgesic activity with percent of inhibition (58.82%, 74.5% and 94.11%) respectively (Fig.1).

Table 1: Effect of aqueous extract of *Pistacia atlantica* Desf. on acetic acid induced writhing in mice.

Treatment groups	Dose mg/kg p.o.	No. Of writhing
Control	0.5 ml/mouse	45±2.58
Aspirin	200	19.6±2.88*
AEPA	200	21±4.61*
AEPA	400	13±1.15*
AEPA	600	3±2*

Values are means ± S.E.M. * P < 0.05, significantly different from control; Student's t-test (n= 6).

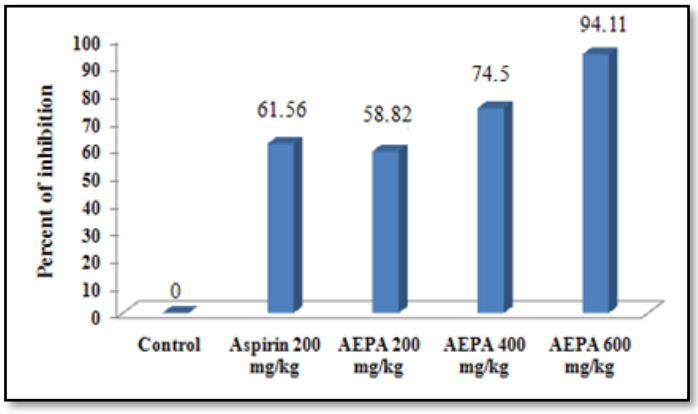

Figure 1: Effect of aqueous extract of *Pistacia atlantica* Desf. and aspirin on acetic acid induced writhing response in mice.

While in tail immersion test result showed significant reduction of pain at 30 min following extract medication at dose 400 mg/kg as compared to control (Table 2).

Table 2: Central Analgesic activity of aqueous extract of *Pistacia atlantica* Desf. by Tail immersion test

	Reaction time in seconds					
Treatment /Dose	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**	**120 min**
Control	1.57±0.13	1.62±0.1	1.63±0.09	1.65±0.13	1.54±0.14	1.45±0.14
Morphine 5mg/kg	1.78±0.19	3±0.15	5.5±0.19	7.49±0.15	5.05±0.14	3±0.13
AEPA 200 mg/kg	1.68±0.3	3.50±0.12	4.15±0.3	3.56±0.27	3.07±0.17	2.63±0.21
AEPA 400 mg/kg	1.94±0.08	3.36±0.04	6.11±0.14*	4.46±0.17	4.18±0.2	2.25±0.26
AEPA 600 mg/kg	1.75±0.34	3.31±0.22	4.50±0.18	4.22±0.11	3.27±0.49	2.78±0.31

Values are means ± S.E.M. *P< 0.05, significantly different from control; Student's t-test (n= 6). AEPA: aqueous extract of *Pistacia atlantica* DESF. Extract.

The results of the present study clearly demonstrated that AEPA possessed a definite dose dependant antinociceptive activity as observed by significant increase in the reaction time in acetic acid induced writhing syndrome, tail immersion test as compared to the control group.

The mechanism of analgesic effect of AEPA could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of piroxicam and other NSAIDs. In other set of experiments, the anti-inflammatory effect of AEPA was evaluated through carrageenan and Trauma -induced inflammation.

Carrageenan and Trauma-induced inflammation in the rat paw represents a classical model of acute inflammation that was used for evaluation of anti-inflammatory activity of drugs or plant extracts [25]. In this study Anti-inflammatory activity of the aqueous extract was measured against acute paw edema induced by carrageenan (Table 3-4). The AEPA in a dose of 200 mg/kg and 400mg/kg showed very good anti-inflammatory activity (93.01% and 88.89% reduction), 1h30 post-medication (maximum inhibitionwere observed during the rest of the experiment at 3hr and 6hr). The standard drug; indomethacin (10 mg/kg) and the AEPA produced significant reduction of carrageenan-induced paw edema as compared to the control rats. The anti-inflammatory activity of all the tested extract was more than that of the standard drug.

Table 3: Effect of aqueous extract of *Pistacia atlantic*a 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	-	0.386±0.01	0.581±0.00	0.478±0.01
Indomethacin	10	0.115±0.003*	0.15±0.006*	0.165±0.007*
AEPA	200	0.016±0.02*	0.023±0.007*	0.016±0.04*
AEPA	400	0.023±0.032*	0.23±0.025*	0.026±0.075*

Values are expressed as mean ± S.E.M. (n = 6), AEPA: aqueous extract of *Pistacia atlantica* Desf.,*P<0.05 statistically significant compared to the control and reference drug (Indomethacin).

Table 4: Percentage of inhibition of inflammation of aqueous extract of *Pistacia atlantica* Desf. using carrageenan-induced rat paw edema.

Treatment groups n=6	Dose mg/kg p.o.	Percent of inhibition of inflammation induced by carrageenan (%)		
		1h30	**3h**	**6h**
Control	-	-	-	-
Indomethacin	10	64.24	74.14	63.59
AEPA	200	93.01	93.06	94.41
AEPA	400	88.89	93.007	90.28

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

On the other hand all doses in the Trauma-induced edema, aqueous extract of *P. Atlantica* showed reduction in paw edema from 1h30 to 6h significantly. The maximum value of percentage of anti-inflammatory activity was (90.59%) after 3h at the dose 400mg/kg next to that (88.08%) for dose of 200mg/kg. The percent inhibition of inflammation by AEPA (200 and 400 mg/kg) was more pronounced than indomethacin (20mg/kg) (Table 5-6).

Table 5: Effect of aqueous extract of *Pistacia atlantica* Desf. on experimental trauma-induced rat paw edema.

Treatment groups n=6	Dose mg/kg p.o.	Mean volume of edema (paw left-paw right) induced by experimental trauma(ml)		
		1h30	**3h**	**6h**
Control	-	0.441±0.01	0.693±0.01	0.563±0.01
Indomethacin	20	0.09±0.006*	0.102±0.008*	0.142±0.006*
AEPA	200	0.11±0.07*	0.15±0.02*	0.18±0.04*
AEPA	400	0.026±0.037*	0.03±0.05*	0.03±0.05*

Values are expressed as mean ± S.E.M. (n = 6), AEPA: aqueous extract of *Pistacia atlantica* Desf., $*P<$ 0.05 statistically significant compared to the control and, reference drug (Indomethacin).

Table 6: Percentage of inhibition of inflammation of aqueous extract of *Pistacia atlantica* Desf. using experimental trauma-induced rat paw edema.

Treatment groups n=6	Dose mg/kg p.o.	Percent of inhibition of inflammation induced by experimental trauma (%)		
		1h30	3h	6h
Control	-	-	-	-
Indomethacin	20	79.55	83.62	75.16
AEPA	200	86.27	88.08	81.04
AEPA	400	90.23	90.59	89.86

N= 6; these results compared with standard drug (Indomethacin, 20mg/kg, p.o.) were administered by the oral route.

The anti-inflammatory activities of the tested plant could be explainedby the abundance of the flavonoids glycosides noted in the aerial parts of *P. atlantica* and the presence of alkaloid in plant extract support the claim that this compound have antinociceptive property since, alkaloid, flavonoids and saponins have been found in other natural product with analgesic and anti-inflammatory properties [26].

CONCLUSION

In conclusion, the study has shown that, AEPA possess effective anti-inflammatory and both central and peripheral antinociceptive activities. On the basis of the present study PA seems to be a promising source of anti-inflammatory analgesic agent as it exhibited its efficacy against all experimental models, this justifying its use in various inflammatory and pain conditions in traditional medicine of Morocco. Anyhow, further studies needed to bring out the active principles and exact mechanism of the extract.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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