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Chemical Composition, Antinociceptive and Acute Toxicity of Pistacia Atlantica Fruit Extract

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ABSTRACT

The goal of the present investigation was to evaluate the antinociceptive activity and acute toxicity of P. atlantica fruit extract in mice. Totally 104 NMRI mice were used in this study. The acute toxicity was evaluated for 2 days. Antinociceptive activity was performed using hot plate, tail-flick, and rotarod test. The LD_{50} values of the P. atlantica fruit extract 1.66 g/kg and the maximum non-fatal doses were 0.93 g/kg. The results revealed that in hot plate and tail-flick tests, P. atlantica fruit extract at the doses of 50, 150 and 350 mg/kg had an analgesic effect as dose-dependent, 30 minutes after administration; so that there was a significant difference between the groups receiving saline and the extract ($p<0.05$). The findings also showed that no significant ($p>0.05$) changes were observed with injecting P. atlantica fruit extract at the doses of 50, 150 and 350 mg / kg in sensory motor test. The results of the current investigation demonstrated the robust antinociceptive activity of P. atlantica fruit extract in mice. However, the exact mechanisms accountable for the pharmacologoical activities remain to be investigated.

Keywords: Analgesic; Pain; GC/MS; Toxicity; Mice

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INTRODUCTION

Nociception is the neural processes of encoding and processing noxious stimuli. This action is launched via nociceptors (pain receptors) that find out mechanical, thermal or chemical changes, higher than the threshold level [1]. Pain is mainly linked with tissue damages or characterized in terms of tissue damages or both. It also might be experienced as numerous types including nociceptive, inflammatory, neuropathic, and functional pains which are caused by various neurobiological mechanisms [2]. Nowadays, it has been proven that pain can affect health and well-being of people, and have negative impacts on relationships, cognitive capability and working skills [3] Based on the World
Health Organization reports, nearly one fifth of the world's populationhas experienced various degrees of chronic pain.

Right now, there is a wide range of chemical drugs which are useful to relieve pain, but they demonstrated remarkable adverse side effects including gastrointestinal bleeding and cardiovascular disorders [4, 5]. Therefore, these reasons have limited their remedial benefits and motivated more drug discovery with high efficacy and low side effects.
Historically, herbal medicines and their derivatives have demonstrated an important alternative for the treatment of illnesses. Pistacia atlantica Desf. (Anacardiaceae family) normally grows in the Mediterranean and Middle Eastern regions particularly in Iran [6]. Traditionally, various parts of P. atlantica, have been consumed to treat gastrointestinal, respiratory, cutaneous, and renal disorders. Besides, in modern medicines, the antioxidant, anti-tumor, anti-asthmatic and antimicrobial properties of P. atlantica have been proven [7].

The goal of the present investigation was to evaluate the antinociceptive activity and acute toxicity of P. atlantica fruit extract in vivo.

MATERIALS AND METHODS

Plant collection

P. atlantica fruits were collected from rural regions of Khorramabad city, Lorestan province, Iran, during September 2016. The plant fruits were recognized by a botanist; additionally, a voucher specimen was placed in the herbarium center.

Preparation of the methanolic extract

One hundered g of the powdered dried fruit was consecutively extracted by the percolation method with 80% methanol for 72 h at room temperature according to a previous study [8].

Gas chromatography/mass spectrometry (GC/MS)

GC/MS analysis was used to determine the main compounds of P. atlantica extract using the solid-phase microextraction (SPME) technique. GC/MS analysis was performed, using the Agilent 6890N, coupled with the HP-5MS column (30m×0.25 mm, film thickness: 0.25 mm). P. atlantica constituents were recognized via matching their relative retention time and mass spectra with the Standard Wiley Library [9].

Acute toxicity test

To determine the acute toxicity, various doses of P. atlantica extract (0.5, 1, 2, and 3 g/kg) were injected as intraperitoneally to mice. The mortality rate was determined after 2 days' administration. LD_{50} values were found by the Probit test in SPSS software [10].

Animals

Male NMRI mice weighing 25-30 g were used in groups of 6 with defined light-dark cycles (12:12-h), in the room temperature (22±2 ºC) and access to food and water, ad libitum. This study was ethically confirmed by the Ethics Committee of Lorestan University of Medical Sciences, Khorramabad, Iran.

Tail flick test

Thermal pain threshold of 30 NMRI mice in five test groups (6 mice per each group; P. atlantica extract at the doses of 75, 150, 300 mg/kg, normal saline, and morphine) was evaluated using tail flick test based on the method described elsewhere [10].

Hot plate test

Here, pain sensitivity of Thirty NMRI mice in five test groups (6 mice per each group; P. atlantica extract at the doses of 75, 150, 300 mg/kg, normal saline, and morphine) was evaluated using an apparatus (LE710 model, Lsi LETICA, Spain) according to the method described by [10].

Rotarod test

Motor coordination was evaluated using the rotarod test according to the method described by [11]. To do this, 30 min after injection of the extract to the animals, they were positioned on the rolling rod, and the falling number of mice during the procedure (3 min) was registered [11].

Statistical analysis

Obtained results have been expressed as the mean ± SEM. SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA) was applied to analyze the obtained data. To estimate the differences between the experimental groups (20), one-way ANOVA with Tukey's post-hoc test was used. And, $P<0.05$ was considered statistically significant.

RESULTS

GC/MS analysis

Table 1 shows the identified constituents of fruit extract from P. atlantica by GC/MS analysis. Totally, 38 components were identified by GC/MS. The main components of fruit extract were β-myrcene (41.4%), α-pinene (32.48%) and limonene (4.66%), respectively.

Table 1. GC/MS analysis of P. atlantica fruit extract

No.	Compounds	Retention time	Components (%)
1.	α-pinene	6.68	32.48
2.	Sabinene	7.85	3.066
3.	β-myrcene	8.33	41.04
4.	α-terpinene	8.72	0.377
5.	Delta-3-carene	8.89	1.337
6.	Limonene	9.51	4.66
7.	Cis-ocimene	9.78	1.621
8.	Trans-ocimene	10.13	1.100
9.	γ-terpinene	10.49	0.478
10.	α-terpinolene	11.47	0.807
11.	Farnesyl acetone	11.69	0.121
12.	Linalool	12.01	1.019
13.	(E)-4,8-dimethyl-1,3,7-nonatriene	12.40	2.390

14.	Alloocimene	12.88	1.363
15.	Trans-pinocarveol	13.34	0.179
16.	Verbenol	13.56	0.582
17.	Pinocarvone	14.11	0.135
18.	L-menthol	14.54	0.133
19.	4-terpineol	14.65	0.165
20.	Piperitone	17.25	0.392
21.	Heneicosane	17.88	0.118
22.	Trans-carane	18.47	0.130
23.	Camphene	19.84	0.085
24.	α-terpinenylacetat	20.30	0.422
25.	α-ylangene	20.96	0.189
26.	Copaene	21.11	0.069
27.	Trans-caryophyllene	22.47	1.368
28.	α-caryophyllene	23.47	0.388
29.	β-santalene	24.15	0.403
30.	Eicosane	24.48	0.140
31.	Isoseychellene	24.64	0.258
32.	α-muurolene	24.75	0.097
33.	Delta-cadinene	25.37	0.228
34.	Nerolidol	26.46	0.683
35.	Verbenyl ethyl ether	26.99	0.292
36.	Mayuron	28.46	0.172
37.	11-n-decyldocosane	30.74	1.339
38.	1-Hexacosene	32.54	0.234
	Total	98.7	

Acute toxicity

The LD_{50} values of the P. atlantica fruit extract were 1.66 g/kg, and the maximum non-fatal doses were 0.93 g/kg.

Tail flick test

As shown in Figure 1, P. atlantica fruit extract showed antinociceptive activity that was dose-dependent. The administration of the P. atlantica fruit extract at the doses of 50, 150 and 350 mg/kg with a mean latency time of 4.5, 5.8 and 7.7 seconds, respectively showed a significant ($p<0.05$) antinociceptive effect compared to the control group (saline recipient). Although, the time latency of the antinociceptive effect of the extract at the dose of 350 mg/kg was more than that of morphine, no significant difference was observed between them ($p>0.05$).

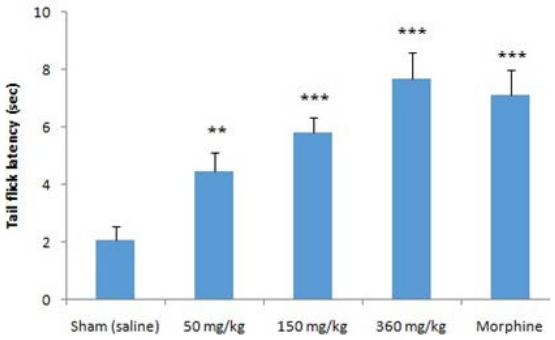

Figure 1. Effect of the P. atlantica fruit extract and morphine on the pain threshold of the mice in the tail-flick test

Hot plate test

The results revealed that P. atlantica at the doses of 50, 150 and 350 mg/kg had an analgesic effect as dose-dependent, 30 minutes after administration (Fig. 2); so that there was a significant difference between the groups receiving saline and the extract ($p<0.05$). Although the reaction time of the antinociceptive effect of the extract at the dose of 350 mg/kg was more than that of morphine, no significant difference was observed between them ($p>0.05$).

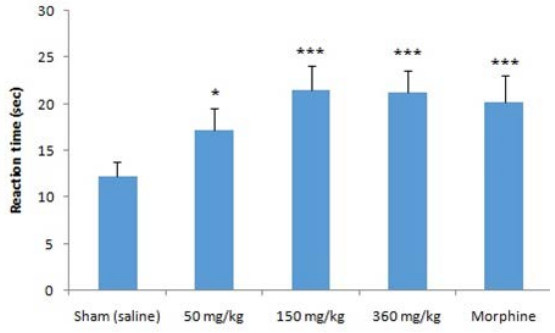

Figure 2. Effect of the P. atlantica fruit extract and morphine on the pain threshold of the mice in the hotplate test

Rotarod test

The results showed that no significant ($p>0.05$) changes were observed with injecting P.

atlantica fruit extract at the doses of 50, 150 and 350 mg / kg in sensory motor test.

DISCUSSION

Nowadays, the chronic inflammatory diseases are considered as one of the main health problems globally; whereas the non-steroid anti-inflammatory drugs are the most common drugs used for the treatment of these diseases [12]. Today, reviews have reported the antinociceptive and anti-inflammatory effects of several herbs including Zataria multiflora, Crocus sativus, Zhumeria majdae, Elaeagnus angustifolia, Urtica pilulifera, and Rosa damascena [13-18]. In the present study, it was aimed to evaluate the antinociceptive activity and acute toxicity of P. atlantica fruit extract in the model of mice. The results demonstrated that P. atlantica fruit extract had antinociceptive activity in both tests (tail flick and hot plate), noticeably and dose-dependently increasing the pain threshold. Regarding antinociceptive activity of plants in Pisatcia genus, [19] have demonstrated that the aqueous and ethanolic extracts of P. vera leaves indicated central and peripheral antinociceptive effects which are dose-dependent, and the central effect might be arbitrated by opioid system. In the current study, it was found that the main components of P. atlantica fruit extract were β-myrcene (41.4%), α-pinene (32.48%) and limonene (4.66%), respectively. Previously, the antinociceptive activities of β-myrcene, α-pinene, and limonene components have been proven in mice [20-22]. Therefore, phytoconstituents in this plant might be responsible for its antinociceptive activity, nevertheless their precise manner of action has been poorly understood.

Due to the fact that the sedative can affect the response to damaging and painful stimuli, the effects of the extract were evaluated using sensory motor test on mice using a rotaroid rod machine. The obtained findings indicated that no remarkable variations were observed with injecting alcoholic extract of wild pistachio with doses of 50, 150 and 350 mg / kg in sensory motor test. Considering the toxicity, the LD50 of the intraperitoneal injection of the P. atlantica fruit extract was 1.66 g/kg, and the maximum non-fatal dose was 0.93 g/kg. Thus, according to the toxicity classification, P. atlantica fruit extract had no significant toxicity against male NIH mice [23].

CONCLUSION

The outcomes of the current study demonstrated the robust antinociceptive activity of P. atlantica fruit extract in mice. However, the exact mechanisms responsible for the pharmacologoical activities remain to be investigated.

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