Research Article

# Anti-inflammatory and Analgesic Activities of *Artemisia absinthium* and Chemical Composition of its Essential Oil

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**ABSTRACT**

Nature has been a source of medicinal agents for thousands of years and has been isolated the number of modern drugs from natural resources. *Artemisia absinthium* used for a variety of medicinal purposes and therapeutic targets in all over the world, such as localized pains, contusion inflammation, anti-rheumatic, include fever reduction, digestive ailments and muscle pain. This study aimed to assess the anti-inflammatory and anti-nociceptive activity of essential oil and aqueous extract from *Artemisia absinthium* for the first time. Chemical compositions of the essential oil were determined by GC/MS. The anti-inflammatory activity was evaluated by carrageenan-induced paw edema in mice. Analgesic activity was assessed by acetic acid-induced writhing, formalin and hot plate tests in mice. Pretreatment with the essential oil (at the dose of 2, 4 and 8mg/kg) and aqueous extract (50, 100 and 200mg/kg) showed potential anti-inflammatory and anti-nociceptive effects to different level. The essential oil at 4 and 8 mg/kg significantly reduced carrageenan induced paw edema. The essential oil and aqueous extract produced significant decreased number of writhing in acetic acid-induced writhing model and increased the response latency in hot plate test after 30 min. Both Essential oil and aqueous extract significantly suppressed in a dose-dependent manner the nociceptive response in the formalin test, while the effect on the late phase was more pronounced. GC–MS analyses showed the presence of twenty components in essential oil. The essential oil and aqueous extract possesses excellent anti-inflammatory activity as well as antinociceptive properties especially peripheral analgesic.

**Keywords:** *Artemisia absinthium*, essential oil, aqueous extract, anti-inflammation, analgesic, GC/MS.

## INTRODUCTION

*Artemisia absinthium* (family: Asteraceae) that commonly known as wormwood (UK), absinthe (France), wermut (Germany) and afsantine (Iran), is a medicinal and aromatic plant. The aerial parts of *A.absinthium* have a long history of use in folk medicine. It is a wild growing plant in Europe, North America, Asia and widely dispersed in north of Iran[1, 2]. All of drugs that use for the management of pain and inflammatory conditions present well known side and toxic effects. Therefore, it is essential that efforts should be made to introduce new medicinal plants to develop effective, cheap and, safer drugs.

The lack of safe and effective analgesic and anti-inflammatory chemical drugs prompted the present study; therefore *Artemisia absinthium* had been selected for study the analgesic and anti-inflammatory activities in indigenous system of medicine. In the previous studies have been reported to possess pharmacological activities of *A. absinthium*; for example: neuroprotective properties[3]; hepatoprotective activity[4], and Antibacterial properties[5]. Also, the ethnopharmacological literature confirms the used of *A. absinthium* for insect bites, muscle pain, heal skin lesions, antiseptic, reducing pain for women during labor, easing the symptoms of depression, and to improve memory[6,7]. The ointment of *A.* absinthium can be used externally to reduce muscle and joint stiffness or pain, and can also help heal bruises[8,9]. The methanolic extract of *A. absinthium* has been reported to protect the liver against chemical toxins[10]. Also, the extracts of this plant has demonstrated to possess a strong antiradical and antioxidant activity in vitro[11], as well as anti-parasitic activity in animal model[12,13]. *A. absinthium* has been reported to enhance the cognitive ability as evidenced by its nicotinic and muscarinic receptor activity in homogenates of human cerebral cortical membranes[14]. Free radical scavenging activity of *Artemisia absinthium* extracts have been reported in both *in vitro* and *in vivo* studies[15,16]. Although much work has been done on this extract plant, but study on its anti-inflammatory and analgesic activities is lacking.

The analgesic and anti-inflammatory activities of alcoholic extracts of *A.absinthium* were evaluated only in India Many year's ago[17], but the biological activity of its essential oil and aqueous extract did not study. Hence, we selected *the A. absinthium* which are used as medicinal remedy by the native people of central region of Iran to confirm the biological activity. The aim of the present study was to investigate the anti-inflammatory and anti-nociceptive properties of *A. absinthium* essential oil and aqueous extract on experimentally induced inflammation and pain. In addition, we determined the chemical composition of essential oil and acute oral toxicity of the essential oil and aqueous extract.

## MATERIALS AND METHODS

### Plant material

The fresh leaves of *Artemisia absintium* were collected at the flowering season in June 2011 from its wild habitat in mountainous area of Semnan City, Iran (35° 32' 40"N, 53° 23' 28"E) (Figure.1). Plant was identified and authenticated as *A. absintium* by experts in the department of herbal medicine of the University of Applied Science and Technology (UAST) Education Center of Semnan, Iran. A voucher specimen (893-4752 – M) of the plant was deposited at the herbarium of the Medicinal Plants Research of UAST.

**Figure 1:** *Artemisia absintium*

### Extraction of the essential oil and aqueous extract

The essential oil of *A. absintium* (EOAA) was isolated by hydrodistillation of the air-dried powdered leaves (100 g) of the plant for 3 h, using a Clevenger-type apparatus, according to the method recommended in European Pharmacopoeia[18]. The volatile distillate was collected over anhydrous sodium sulphate and kept in airtight containers prior in refrigerated until further analysis. The yield of the oil was 2.8% (v/w), based on dry plant weight. For preparing the aqueous extract *of A. absinthium* (AEAA), fresh green leaves were separated and cleaned, then dried in shade at room temperature and powdered mechanically. The aqueous extract was prepared by macerating 500 g of dried leaves in distilled water for 24 h and then the extract was filtered and lyophilized and the dried powders were used for experiment. The yield obtained was 9.7%. The aqueous extract was stored at 4 ◦C until used.

### Experimental animals

Albino mice (25–30 g) were purchased from pasture institute (Tehran, Iran). The animals were kept in standard environmental conditions (21°C, 60–70% humidity) with well-ventilated. The animals were housed in standard cages at a room temperature of 23±1°C with a 12 h light/dark cycle with free access to standard diet (standard laboratory rodent's chow) and water. Male albino mice were used for analgesic (Hot-plate, formalin and writhing) and anti-inflammatory (carrageenin-induced mice hind paw edema) tests; also female and male mice were used for acute toxicity assay. The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research[19].

### Drugs and chemicals

The following drugs and reagents were used: Carrageenan, Morphine, Aspirin, and Naloxone, which were purchased from Sigma (Sigma Chemical Company, St. Louis,USA); and formaldehyde that was from Merck (Darmstadt, Germany). Acetic acid was purchased from Sinopharm Chemical Reagent Co., Ltd.

### Chemical analysis of the essential oil

The oil was analyzed by GC and GC/MS. GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with a flame ionization detector (FID) detector and a fused silica capillary column DB-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness ). The oven temperature was programmed at 60°C (4 min), and then rising to 300°C at 4°C/min. Other operating conditions were as follows: Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector and detector temperature were kept at 250 °C and 300 °C, respectively. Volume of injected samples was 0.5µl. The split ratio was 1:50. The MS operating parameters were as follows: ionization potential, 70 ev; ionization current, 2 A; inlet and ionization source temperatures were 320 and 300°C, respectively; resolution, 1000; scan rate was 0.34 s per scan. Identification of components in the oil was based on GC retention indices relative to *n*-alkanes and computer matching with the Wiley 275 L library as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature[20,21,22]. The relative percentage of the oil constituents was calculated from the GC peak areas.

### Acute toxicity test

The median lethal dose (LD50) of the essential oil and aqueous extract were evaluated in mice according to the modified method of Lorke[23]. The animals were handled in accordance with international principles guiding the use and handling of experimental animals[19]. All the animals were randomly divided into five groups; one control group and four treated groups, containing four animals per group. Groups 1 and 2 were orally administered 50 and 100 mg/kg body weight Essential oil and Groups 3 and 4 were orally administered 700 and 1400 mg/kg body weight aqueous extract. Group 5 (Control group), having saline solution (0.9%) 10 ml/kg. The mice in test groups were then allowed free access to food and water. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity,

decreased body/limb tone, decreased respiration and death. They were observed for 24 hours for signs of toxicity. Also, animals in the different groups were observed for 2 h post treatment for immediate signs of toxicity and the mice in test groups observed over a period of 7 days for signs of behavioral changes and toxicity signs. The number of deaths within this period of time was recorded. Log–dose response plots are constructed for the plant extract, from which the median lethal dose (LD50) of the EOAA and AEAA were determined.

**Pharmacological studies**

***Analgesic activity***

The central analgesic activity of the EOAA and AEAA was studied against thermal stimuli using the hot plate test, while peripheral analgesic activity of these agents was evaluated using the acetic acid-induced writhing test. Also, the formalin test performed for the study both central and peripheral analgesic effects in its first (early) and second (late) phases.

***Acetic acid-induced writhing test***

Analgesic activity was evaluated according to the method of Koster et al.[24], and assessed by the acetic acid abdominal constriction test in mice (writhing test). The abdomen writhing is a model of visceral pain and was produced by intraperitoneal (i.p) administration of acetic acid solution (1%, 10 mL/kg) to each mouse. Mice were divided into 8 groups (n= 6). Group 1 served as control administered with 10 ml/kg distilled water intra-peritoneal, groups 2, 3 and 4 received 2, 4 and 8 mg/kg of essential oil and groups 5, 6, and 7 received 50, 100 and 200 mg/kg aqueous extract orally respectively. Group 8 served as positive control and was treated orally with 300 mg/kg Aspirin. 30 min after the administration of different agents, mice in all groups were treated with acetic acid. Five minutes after administration of acetic acid mice were placed in individual cage, and the number of writhes was counted for each mouse during 30 min. Antinociceptive activity was expressed as inhibition percent of the usual number of writhes observed in control animals. The percentages of inhibition were calculated according to the following formula:

% inhibition = ((number of writhes) control – (number of writhes) treated group) × 100/(number of writhes)control.

**Formalin test**

The formalin test was carried out as described by Dubuisson and Dennis[25]. This test possesses two distinctive phases, possibly reflecting different types of pain. The formalin (20 ml of 2.5%) was injected subcutaneously into the plantar surface of the left hind paw of the mice. One hour before testing, the animal was placed in a standard cage (30×12×13 cm), that served as an observation chamber. Each animal was tested once only. Mice were divided into 8 groups (n = 6). Group 1 served as control administered and received only saline (10 mg/kg). The EOAA was given orally to groups 2, 3 and 4 (2,4 and 8 mg/kg), and groups 5, 6 and 7 received AEAA (50,100and 200 mg/kg) orally as a treatment groups, respectively. Morphine (10 mg/kg) was administered intraperitoneal (i.p) to group 8 to serve as positive control. The drugs were administered 30 min before injection of formalin. The behavioral responses to nociception including biting, licking and scratching of the injected hindpaw were noted and the time spent was recorded to 60 min. The first 5 min post formalin injection is known as the early phase (between 0 and 5 min) and the period between 15 and 60 min as the late phase.

**Hot-plate test**

The hot plate-induced pain test was carried out on groups of adult mice using a hot plate apparatus (Electrothermal Eng. Ltd.), maintained at 55 ± 0.5 °C[26]. The paws of mice are very sensitive to heat at temperature, which are not damaging the skin. The animals (n= 6) were placed on glass funnels in the heated surface. The time between placement of the animal on the hot-plate and the occurrence of either the licking of the hind paws, shaking or jump off from the surface was recorded as response latency. Before the experiments, all animals were tested for heat stimulation latency and the mice with baseline latencies of more than 10s were eliminated from the study 24 h later. A cut off period of 15 sec, was observed to avoid damage of the paw. All animals were observed before (0) and 30, 30, 45, 60, 90 and 120 min after all agent administration. Animals were divided into 8 groups of seven mice each; the 1st group was orally administered with oral distilled water as a control group while in the 2nd group (positive control) morphine (10 mg/kg, i.p.) was used as a reference drug. In the groups 3 to 8, animals were orally given the EOAA (2,4 and 8 mg/kg) and AEAA (50,100and 200 mg/kg), respectively.

***Anti-inflammatory activity***

In vivo anti-inflammatory activity was evaluated on the basis of inhibition of carrageenin-induced by the injection of carrageenan (an edematogenic agent) into the sub-plantar region of the right hind paw of the mice according to the method described by Winter et al[27]. Before any treatment, the average volume (three or four measurements) of the right paw of each animal was determined using a plethysmometer (Ugo Baseil no. 7140). Mice were divided into different groups of 6 animals per group. Mice in groups 1 served as control group administered with saline, and mice in group 2 (standard group) received aspirin (300 mg/kg, p.o.) as the reference drug. Groups 3 to 5 were treated with EOAA (2, 4 and 8 mg/kg), and AEAA was administered orally (2, 4 and 8 mg/kg) in groups 6 to 8 as test groups. One hour after oral administration of the various agents (EOAA, AEAA, aspirin and saline) oedema was induced by an injection of 0.1ml of carrageenan (1%, w/v in saline). The paw volumes of these mice were measured using a plethysmometer. The measures were determined at 0 h

(V0: before edematogenic agent injection) and 1, 2, 3, 4 and 5h intervals later (VT). The difference between VT (1, 2, 3, 4 and 5 h) and V0 was taken as the edema value. The percentages of inhibition were calculated according to the following formula:

% inhibition = ((VT –V0) control – (VT –V0) treated group) × 100 / (VT –V0) control.

### Statistical analysis

Results obtained were expressed as mean±SEM. The data were analyzed using one way ANOVA followed by Tukey's and Dunnett's multiple comparison tests. Results with $p < 0.05$ were considered statistically significant.

## RESULTS

### Phytochemical screening

The plant leaves yielded 1.1% of a pale-yellowish essential oil with a fresh pleasant odor. In the essential oil extracted from *A. absinthium* 20 compounds were identified, representing 98.02% of the total oil components detected, which are listed in Table 1 with their percentage composition and retention indices. The main constituents in the *A. absinthium* leaf essential oil were Nerolidol (49.91%), Santolina triene (15.58%), α-pinene (6.99%) *trans*-β-Farnesene (4.95%).

**Table 1:** Chemical composition of *Artemisia absinthium* essential oil

| No. | Compound | RI | Percentage |
|---|---|---|---|
| 1 | Santolina triene | 917 | 15.85 |
| 2 | α-Pinene | 921 | 6.99 |
| 3 | 1-Butanol | 931 | 1.86 |
| 4 | 2-β-Pinene | 980 | 0.87 |
| 5 | dl-Limonene | 986 | 1.04 |
| 6 | 1,8-Cineole | 995 | 0.73 |
| 7 | Santolina triene | 1009 | 0.59 |
| 8 | *trans*-Photonerol | 1012 | 4.49 |
| 9 | 4-Hexen-1-ol | 1016 | 1.09 |
| 10 | α-Terpineol | 1035 | 0.84 |
| 11 | Lyratyl acetate | 1046 | 0.78 |
| 12 | Benzenemethanol | 1051 | 0.61 |
| 13 | *trans*-β-Farnesene | 1058 | 4.95 |
| 14 | δ-Cadinene | 1062 | 0.53 |
| 15 | Nerolidol | 1082 | 49.91 |
| 16 | Caryophyllene oxide | 1098 | 0.87 |
| 17 | 1H-Benzocycloheptene | 1159 | 0.58 |
| 18 | Eudesma-4(14) | 1172 | 0.58 |
| 19 | En-in-dicycloether | 1190 | 4.26 |
| 20 | Benzene | 1259 | 0.60 |
| | Total | | 98.02 |

### Analgesic tests

The results demonstrated the significant antinociceptive activities of the EOAA and AEAA. Their antinociceptive activities were the comparable with the reference drug (Aspirin and Morphine). The EOAA has the higher anti-nociceptive activity in comparison with AEAA.

### Acetic acid-induced writhing

The peripheral analgesic effects of the essential oil and aqueous extract were evaluated by the acetic acid-induced abdominal writhing test in mice. The analgesic effect was tested for concentrations ranging from 2 to 8 mg/kg of EOAA and 50 to 200 for AEAA. The results (table 2) showed that both EOAA and AEAA caused an inhibition on the writhing response induced by acetic acid with potency comparable to the reference drug, Aspirin ($p < 0.05$). Doses of 4 and 8 mg/kg of the EOAA significantly ($P < 0.05$) inhibited the writhing response induced by acetic acid by 82.31% and 94.68% respectively, and dose of 8 mg/kg of the AEAA significantly ($P < 0.05$) inhibited the writhing response by 88.41%, whereas aspirin at a dose of 300 mg/kg exhibited 81.23% inhibition. The AEAA could not exert a significant decrease of abdominal twitches at doses of 50 and 100 mg/kg. EOAA and AEAA dose-dependently reduced the intensity of acetic acid-induced abdominal constriction in mice.

**Table 2:** Effect of *Artemisia absinthium* essential oil and aqueous extract on acetic acid-induced writhing in mice

| Groups | Dose (mg/kg) | Number of writhes | Inhibition of writhing (%) |
|---|---|---|---|
| Control | - | 37.6 ± 4.17 | - |
| EOAA | 2 | 22.7 ± 2.36 | 36.49 |
| | 4 | 5.3 ± 3.24* | 82.31 |
| | 8 | 2.4 ± 3.16* | 94.68 |
| AEAA | 50 | 21.3 ± 5.26 | 36.19 |
| | 100 | 9.8 ± 3.07 | 62.07 |
| | 200 | 5.7 ± 1.29* | 88.41 |
| Aspirin | 300 | 7.4 ± 3.16* | 81.23 |

EOAA: Essential oil of *Artemisia absinthium*; AEAA: Aqueous extract of *Artemisia absinthium*; Values are mean ± SEM (n = 6); * $P < 0.05$; significant from control group.

### Formalin test

The effects of EOAA and AEAA in early and late phases of the formalin test are shown in Table 3. Early (first) and late (second) phases are corresponded to neurogenic and inflammatory pains respectively. EOAA and AEAA don't show significant activity in early phases (0-5 min) and possessed only mild inhibitory effect on licking response. However, the EOAA and AEAA in a dose-dependent manner inhibited paw licking of late phase (15–60 min) significantly ($P<0.001$). The standard drug, Morphine (10 mg/kg), significantly reduces the pain responses of the early and late phases with inhibitions of 91% and 95%, respectively (Table 3). The greatest effects (92% and 91% inhibition in EOAA and AEAA respectively) were produced at the highest dose (8 mg/kg in EOAA and 200 mg/kg for AEAA) in late phase that were comparable to standard drug.

**Table 3:** Effect of *Artemisia absinthium* essential oil and aqueous extract on the early phase and late phase of formalin test in mice

| Groups | Dose (mg/kg) | First phase (0-5 min) | | Second phase (15-60 min) | |
|---|---|---|---|---|---|
| | | Paw licking time (s) | Inhibition (%) | Paw licking time (s) | Inhibition (%) |
| **Control** | - | 45.16 ± 2.27 | - | 20.53 ± 4.38 | - |
| **EOAA** | 2 | 39.45 ± 5.21 | 16 | 4.37 ± 2.36*** | 84 |
| | 4 | 15.32 ± 1.16** | 70 | 4.56 ± 1.29*** | 87 |
| | *8* | 6.54 ± 2.35 *** | 89 | 3.12 ± 1.41*** | 92 |
| **AEAA** | 50 | 35.26 ± 1.18 | 19 | 9.05 ± 1.24*** | 69 |
| | 100 | 32.41 ± 2.26 | 21 | 6.19 ± 2.35*** | 75 |
| | 200 | 30.37 ± 2.41 | 23 | 3.26 ± 0.27*** | 91 |
| **Morphine** | 10 | 6.25 ± 2.67*** | 91 | 2.13 ± 0.12*** | 95 |

EOAA: Essential oil of *Artemisia absinthium*; AEAA: Aqueous extract of *Artemisia absinthium*; Values were expressed as mean ± S.E.M. (n = 6); $^{**}P < 0.01$; $^{***}P < 0.001$ compared with control group.

### Hot plate test

To check for central anti-nociceptive effects of the *A. absinthium*, the hot-plate test carried out, and the results are shown in Table 4. The EOAA (2, 4 and 8 mg/kg) and AEAA (50, 100 and 200 mg/kg) significantly increased the reaction time of mice after 30 min treatment as compared to the control groups. There was a dose-independent increase in response to thermal stimulation compared with control mice. The EOAA has more potent as an anti-nociceptive agent than AEAA in most cases (Table 4). These effects were comparable to that produced by standard drug (Morphine; 10 mg/kg).The study also shows that the Morphine significantly delayed the reaction time of test.

### Carrageenan-induced oedema test

The anti-inflammatory effects of the EOAA and AEAA on carrageenan-induced paw edema and percentages of inhibition in mice are shown in Table 5.

**Table 4:** Effect of *Artemisia absinthium* essential oil and aqueous extract on analgesic activity in hot-plate test

| Groups | Dose (mg/kg) | Reaction time (s) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 min | 15 min | 30 min | 45 min | 60 min | 90 min |
| **Control** | - | 8.12 ± 1.23 | 6.05 ± 0.21 | 7.36 ± 1.42 | 8.05 ± 0.19 | 8.25 ± 0.16 | 7.31 ± 0.13 |
| **EOAA** | 2 | 6.32 ± 0.24 | 6.04 ± 0.16 | 11.20 ± 1.13* | 7.14 ± 0.16 | 11.02 ± 1.27* | 6.23 ± 0.25 |
| | 4 | 7.40±0.54 | 13.12 ± 1.10* | 8.28 ± 0.14 | 12.04 ± 1.53* | 16.14 ± 1.34* | 27.35 ± 3.17* |
| | 8 | 6.10 ± 0.45 | 10.28 ± 0.15* | 9.05 ± 0.16 | 26.54 ± 2.41* | 9.06 ± 1.17 | 12.26 ± 1.19* |
| **AEAA** | 50 | 6.15 ± 0.29 | 5.23 ± 0.19 | 8.05 ± 1.14 | 8.12 ± 0.17 | 9.24 ± 0.16 | 8.06 ± 0.31 |
| | 100 | 6.13± 0.15 | 6.48 ± 0.03 | 8.07 ± 1.02 | 9.16 ± 1.18 | 12.10 ± 1.23* | 12.15 ± 1.36* |
| | 200 | 8.06±0.29 | 11.02 ± 0.31* | 9.36 ± 1.04 | 17.09 ± 1.15* | 9.06 ± 0.23 | 10.14 ± 0.27 |
| **Morphine** | 10 | 6.3±2.17 | 7.14 ± 0.42 | 8.19 ± 1.06 | 9.12 ± 1.24 | 14.25 ± 1.53* | 19.37 ± 2.61* |

EOAA: Essential oil of *Artemisia absinthium*; AEAA: Aqueous extract of *Artemisia absinthium*; Values are expressed as mean ± SEM (n = 6); *$P < 0.05$ compared to corresponding control.

**Table 5:** Influence of *Artemisia absinthium* essential oil and aqueous extract on carrageenan-induced mice hind paws oedema

| Groups | Dose (mg/kg) | Inflammation (%I) ± SEM | | | | |
|---|---|---|---|---|---|---|
| | | 1h | 2h | 3h | 4h | 5h |
| Control | - | 37.24 ±03.16 | 47.03 ± 1.25 | 48.29 ± 1.07 | 42.15 ± 0.13 | 37.31 ± 1.16 |
| EOAA | 2 | 30.45 ± 1.24 $^{c}$ (17.72) | 38.26 ± 1.72 $^{c}$ (16.87) | 43.31 ± 1.06 $^{b}$ (13.74) | 24.42 ± 1.12 $^{a}$ (46.52) | 21.32 ± 1.09 $^{a}$ (48.51) |
| | 4 | 28.19 ± 1.82 $^{b}$ (24.76) | 36.29 ± 1.87 $^{b}$ (21.58) | 30.76 ± 1.09 $^{a}$ (39.24) | 22.18 ± 1.25 $^{a}$ (52.21) | 18.29 ± 1.06 $^{a}$ (55.37) |
| | 8 | 21.36 ± 1.17 $^{a}$ (44.28) | 29.53 ± 1.07 $^{a}$ (36.54) | 27.19 ± 1.18 $^{a}$ (46.82) | 20.85 ± 1.02 $^{a}$ (55.27) | 19.16 ± 0.12 $^{a}$ (53.72) |
| AEAA | 50 | 33.06 ± 1.18 $^{c}$ (12.54) | 42.33 ±0.21 (10.19) | 42.12 ± 1.36 $^{c}$ (13.15) | 36.07 ± 1.09 $^{c}$ (15.22) | 31.16 ± 1.34 $^{c}$ (19.65) |
| | 100 | 32.27 ± 0.17 $^{c}$ (14.28) | 40.25 ± 0.26 (11.34) | 40.56 ± 1.25 $^{c}$ (13.78) | 35.42 ± 1.46 $^{b}$ (17.41) | 30.09 ± 1.24 $^{b}$ (20.18) |
| | 200 | 30.24 ± 1.28 $^{c}$ (17.26) | 37.42 ± 1.05 $^{c}$ (18.35) | 38.54 ± 1.20 $^{c}$ (15.32) | 34.06 ± 1.25 $^{b}$ (19.06) | 28.47 ± 1.29 $^{c}$ (21.62) |
| Aspirin | 300 | 21.4 ± 1.14 $^{a}$ (42.36) | 29.12 ± 1.09 $^{a}$ (36.02) | 33.64 ± 1.16 $^{a}$ (33.12) | 25.18 ± 1.06 $^{a}$ (45.23) | 20.15 ± 1.08 $^{a}$ (50.64) |

The anti-inflammatory effects (%A) of the essential oil and aqueous extract on carrageenan induced inflammation are indicted in parenthesis. I: Inhibition; Each value represents the mean±SEM (n = 6); EOAA: Essential oil of *Artemisia absinthium*; AEAA: Aqueous extract of *Artemisia absinthium;* $^{a}$ $p < 0.001$, statistically significant relative to control; $^{b}$ $p < 0.01$, statistically significant relative to control; $^{c}$ $p < 0.05$, statistically significant relative to control.

The EOAA and AEAA exhibited varying degrees of anti-inflammatory activity. The EOAA has the high potential of anti-inflammatory at all doses and significantly reduced carrageenan induced paw edema in mice ($p < 0.001$), but the AEAA has the moderate activity.

The maximum volume of paw edema (48.29 ± 1.07 ml) in mice increased progressively and reached its maximum after 3h of carrageenan injection in control group. The highest anti-inflammatory activities were observed after 4 and 5 h for EOAA (55.27% and 55.37% respectively) of carrageenan injection, and 5 h after injection for AEAA (21.62%) (Table 5). The EOAA, in high dose (8 mg/kg) had a considerable anti-inflammatory effect in all time of test and this effect was maintained for 5h post injection of carrageenan. The highest inhibition of edema for Aspirin (300 mg/kg) produced 5h after injection of carrageenan. The anti-inflammatory effect of 4 and 8 mg/kg of EOAA were comparable to the standard drug (aspirin) (Table 5).

## DISCUSSION

This is the first study evaluating the in vivo acute toxicity, antinociceptive and anti-inflammatory activities of the essential oil and aqueous extract of *Artemisia absinthium*. In order to investigate for possible central anti-nociceptive activity of the herb, the hot-plate test was performed, because it had several advantages, particularly the sensitivity to strong analgesics and limited tissue damage. In relation to mechanism of drugs in hot plate, the previous studies were speculated that it may be linked to processes involved in the prevention of sensitization of the nociceptor, and/or inhibition of central pain receptors[24,28]. Also, this test indicates narcotic involvement[29], with opioid receptors. Using the hot plate thermal stimulation indicates that EOAA and AEAA have central anti-nociceptive (thermal reaction time prolongation) effects against this model. The EOAA was the most potent central anti-nociceptive and increased the pain threshold to hot-plate in mice, while AEAA demonstrated the moderate central anti-nociceptive activity compared with control group. The results from hot plate test indicated that EOAA significantly increased the latency of jumping response when treated at 4 and 8 mg/kg. In this analgesic testing model, the standard drug (Morphine, 10 mg/kg) prolonged the reaction time of the animals. Taken together, the data presented demonstrate that the EOAA and AEAA induce variable degrees of central analgesic effects. These results can provide useful information for using from *A. absinthium* as a source of analgesic drugs. The acetic acid-induced abdominal constriction method is the animal model typically used to evaluation peripheral activity of agents[30,31]. Acetic acid itself may cause pain; at the same time, it can also stimulate the tissue to produce several mediators such as histamine, serotonin, ytokines, and eicosanoids with an increase in peritoneal fluid levels of these mediators[32,33]. Therefore, antinociceptive activity of drugs may be related to the reduction in the liberation of those inflammatory mediators or cyclo-oxygenases and/or lipoxygenases, and or by direct blockage of receptors resulting in peripheral anti-nociceptive effects[23,34]. This method has been associated with prostanoids in general, e.g. increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipooxygenase products[35]. Prostaglandins induce abdominal constriction by activating and sensitizing the peripheral chemo-sensitive nociceptors[36], which are mostly responsible for causing inflammatory pain[37]. In treatment groups, that administered EOAA (2, 4 and 8 mg/kg) and AEAA (2, 4 and 8 mg/kg), there was a dose-dependent decrease the abdominal constriction response compared with control mice. This observation indicates that the EOAA and AEAA had significant peripheral analgesic properties. Aspirin (300 mg/kg), used as a positive control, also inhibited the writhing response. The formalin test has two specific phases, possibly reflecting different types of pain. This test is capable of discerning between nurogenic pain (early phase, acute, non-inflammatory and CNS modulated) and inflammatory (chronic and peripheral pain)[38,39]. The early phase reflects to be due to direct effect of formalin on nociceptors, whereas the second phase is dependent of peripheral inflammation[40,41]. The formalin test also determined the herb's potential on chronic inflammation compared to other test such as acetic acid, hotplate and tail immersion, which are indicative of acute pain[42]. Substance P and bradykinin participate in the neurogenic phase, and this pain is caused by direct chemical stimulation of nociceptive afferent fibers (predominantly C fibers) which can be suppressed by opiate like morphine[43]. The inflammatory pain (second phase) is caused by the release of inflammatory mediators like nitric oxide, histamine, prostaglandins, bradykinin, serotonin in the peripheral tissues[36;44], and from functional changes in the spinal dorsal horn[45]. The formalin test normally determined the site and mechanism of action of the agents[38].This test showed that the both EOAA and AEAA had significant peripheral analgesic activity (inhibition of inflammatory pain) rather than central analgesic effect that was moderate (inhibition of non-inflammatory pain). Results indicated the little difference between analgesic activities at all doses of EOAA and AEAA to morphine (10 mg/kg) as standard drug in second phase. In this study, *A. absinthium* was investigated for potential of anti-inflammatory to the carrageenan induced paw edema; and for analgesic activity using the acetic acid-induced writhing, formalin, and hot plate tests. The carrageenan induced mice paw edema selected for searching of anti-inflammatory activity of *A. absinthium*. Inflammation induced by carrageenan is an acute and highly reproducible inflammatory model. Carrageenin paw edema is a test used largely to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators[46]. This suitable test also has frequently been used to access the anti-edematous effect of natural products[47]. The carrageenin-induced inflammatory process in the mice involves two phases (biphasic event) through sequential release of several mediators. The early phase (1-2 hours ) hyperemia being due to the release of

histamine and serotonin and increased synthesis of prostaglandins in the damaged paw tissues; and the delayed edema is sustained by prostaglandin release and is also mediated by bradykinin, leukotrienes, poly-morphonuclear cells, and prostaglandins produced by tissue macrophages[48]. The EOAA exhibit significant anti-inflammatory activity against carrageenan, and the AEAA has moderate activity compared with the control group. A marked inhibition of edema formation was observed at the third, fourth and fifth hour with all doses of EOAA in a dose-dependent manner. The dose of 8 mg/kg of the EOAA showed an inhibitory at all test times. This anti-inflammatory response was also significant in mice pretreated with Aspirin. These results suggest that the mechanism of anti-inflammatory activity of EOAA may be associated with synthesis or release of inflammatory mediators such as the histamine, serotonin, bradykinins and prostaglandins. But, all doses of AEAA showed a gentle decrease in the percentage of paw edema in all time after injection. Toxicological studies showed that EOAA and AEAA are safe at the effective doses, since the test of acute toxicity showed that the EOAA and AEAA produced toxic activity only at high doses (100mg/kg in EOAA and 1400 mg/kg for AEAA). In other words, it is important attention to that the toxic doses are much higher than effective anti-inflammatory and analgesic doses in mice (4 and 8 mg/kg). This finding suggests that the EOAA and AEAA are safe in mice. More than 65% of the essential oil components of *A. absinthium* were due to a Nerolidol and Santolina triene. In the previous study, the chemical composition of *A. absinthium* oil from Turkey, were investigated. Comparing our results with it study shows that the main components are different to our study; trans-Sabinyl acetate (24.6%), Myrcene (10.8%), trans-Thujone (10.1%) and Linalool (4.6%) were main constituent in their research[49]. The essential oil was composed mainly of monoterpenes and sesqui-terpenes, of which Nerolidol (49.91%), Santolina triene (15.58%) made up 65.49% of the essential oil. The other identified components made up 32.61% of the essential oil (Table 1).

## CONCLUSION

In conclusion, this study has shown that the essential oil and aqueous extract of *A. absinthium* possess significant anti-inflammatory and antinociceptive effects in laboratory animals at the doses investigated. The results suggested that the EOAA and AEAA have the CNS-modulated effect in pain inhibition, based on three different *in vivo* models. Their peripheral analgesic activity was significant and they have been also repeatedly confirmed by two *in vivo* models. The essential oil showed the more analgesic and anti-inflammatory activity than aqueous extract. The anti-inflammatory effect of aqueous extract may be due to its flavonoids. The results support a rational basis for the traditional use of *A. absinthium* in some painful and inflammatory conditions. The further studies are required to clarify the complete pharmacological profile and mechanism of analgesic and anti-inflammatory activity of *A. absinthium*. Also further phytochemical and biological tests are suggested to determine the active chemical constituent(s) responsible for these activities.

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