http://www.ljmr.com.ly/ eISSN:2413-6096

Original Article

Analgesic and anti-inflammatory activity of Arbutus pavarii ethanoic extract in albino rats and mice

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ABSTRACT

Background: Arbutus pavarii; locally known as "Shmeri", is an endemic plant of Libya, it is mainly grown in El-Jabal El-Akhdar region. In traditional medicine, leaves and bark are used extensively as a remedy for gastritis, renal infections, constipation, and various of other human aliments. Furthermore, A. pavarii leaves showed some important biological activities, for example; it has hypoglycemic, antioxidant, antimicrobial, and cytotoxic activities. Moreover, previous phytochemical studies showed the presence of phenols, flavonoids, carbohydrates, glycosides, sterols, triterpenes, and tannins. Objective: The present study aimed to investigate the analgesic and anti-inflammatory activities of A. pavarii leaves extract (APLE). Methods: fresh leaves of A. pavariiwere collected on march 2021. Leaves were shad-dried, ground to a fine powder and macerated in ethanol and evaporated under reduced pressure in rotary evaporator. The crude extract was administered by oral gavage at a dose of 30%. The analgesic activity was examined by hot plat method in albino rats, and by the acetic acid induced writhing response in albino mice. The anti-inflammatory effect was evaluated by both carrageenan and formalin-induced paw oedema in rats. Results: The ethanoic extract of leaves significantly increased the mean reaction time in the hot plate test (◉<0.001). Also, it significantly decreased the mean of writhes induced by acetic acid(◉<0.0001). In addition, the anti-inflammatory tests by both formalin and carrageenan showed a significant decrease in the paw volume of treated rats in comparison to the control($p<0.001$). Conclusion: These results indicate that ethanolic extract of A. pavarii leaves may contain bioactive component(s) that have analgesic and anti-inflammatory activities.

Key words: *Arbutus pavarii*; carrageenan; hot plate test; writhing response.

Citation.Saad.Shaban E.**Analgesic and anti-inflammatory activity of Arbutus pavarii ethanoic extract in albino rats and mice**
2022;16(2):https://doi.org/10.54361/ljmr.16211
Received: 10/05/22**accepted**: 20/06/22; **published**: 31/12/22

Introduction

Folk medicine has been widely used for hundreds of years as a treatment for many diseases. Plants have been the most generous source of bioactive compounds that able to cure several diseases. According to estimates from the World Health Organization (WHO), almost 80% of people worldwide rely on herbal therapies for some part of their basic medical needs [1].In addition,natural products derived from plants are good source for the isolation and synthesis of important therapeutic agents [2].Alkaloids, flavonoids, phenolic compounds and tannins have been established as the most important phytochemical compounds of medicinal plants [3]. Therefore,numerous studies are conducted worldwide to examine and assess the chemical makeup, biological activity, effectiveness, therapeutic applications, and toxicity of medicinal plants [4]. Arbutus is a genus of about 12 species of flowering plants that belongs to the Ericaceous family (Health family). Traditionally some genera of Ericaceae are extensively employed in the treatment of arterial hypertension, headache, constipation, urinary tract infections, and arthritis, and also used as antiseptic and diuretics [5].

Arbutus pavarii; locally known as "Shmeri", is an endemic plant of Libya, it is mainly grown in El-Jabal El-Akhdar region. It is one of the most extensively used endemic plants [6]. A. pavarii leaves and barks have been traditionally used by Libyan people as a remedy for gastritis, renal infections, cold, tuberculosis and various other human aliments[6]. Also, it has some important biological activities such as, antimicrobial, antioxidant, anticancer (7,8,9,10and hypoglycemic effect [11].

Phytochemical studies revealed the presence of several bioactive compounds such as phenols, flavonoids, carbohydrates, glycosides, unsaturated sterols, triterpenes, and tannins [12]. Furthermore, Previous phytochemical studies on leaves described the presence of arbutin, α-amyrin, lupeol, oleanolic acid, catechin, isoquercitrin, myricetin, gallic and ferulic acid as the major constituents [13]. However, the published data regarding A. pavarii plant are scarce, and to our knowledge, there is no publisheddatadealing with the analgesic and anti-inflammatory effects of A. pavarii

leaves. Therefore, the analgesic and anti-inflammatory activity of A. pavarii plant were investigated in this study.

Materials and methods

Instruments

Instruments used in this study are a Rotary evaporator (Stuart RE300), digital analytical balance (mettle Toledo AE 240), digital plethysmometer (Ugo Basile, cat no 7140, Italy), hot plate (Ugo Basile, cat no 35100, Italy), filter paper (whatman, cat no 1001185), syringes with needles, and feeding tubes for rats and mice.

Chemicals and drugs

Chemicals and drugs used in this study includedethanol 96.6% (AYATAS,Turkey), Glacial acetic acid (PDH laboratory supplies, England), formalin (Bio-Optica, Italy) and carrageenan (Sigma-Aldrich, Denmark). All other chemicals and solvents used in the present investigation are of analytical grade.

Collection, identification and preparation of plant

The fresh maturated leaves of A. pavariiwere collected in march 2021, from EL-Jabal EL-Akhdar, El-Bieda city, Libya.The plant under investigation wasidentified and authenticatedby the Department of Botany, Faculty of Sciences, Tripoli University, Preparation of extract

1000 g of the powdered leaves were macerated in 3 litres of ethanol (96.6%) for 3 days. After 3 days with continuous agitation, the extract was filtered, and the marc was re-macerated twice using the same volume ofethanol. The crude extract was then concentrated at 40˚C under reduced pressure by rotary evaporator. The residue left after evaporation of ethanol was weighed and stored at -20˚C until further use[14], [15].

Experimentalanimals

Healthy adult Albino rats (180–250 gm) and adultAlbino mice (22–29gm) were obtained from the Animal House Unit (AHU) ofthe Libyan Center for Medical Research, Zawia. The animals were kept under standard housing conditions, they were housed in groups of six per standard cage, under a 12:12 h light/dark cycle at 25±2˚C with free

access to foodand water at least two weeks before the experiment. All protocols used in this study were approved by the Departmental Ethical Committee of the Faculty of Pharmacy, University of Tripoli.

Study design

Two groups of animals (mice or rats) were used, each group consisting of six. The first group received vehicle (gum acacia suspension 5%, 2ml/100g, P.O) and served as a control group. While the second group was given the extract (30% w/v of A. pavarii residue in gum acacia, 2ml/100g, P.O), and served as a test group.

Acute oral toxicity study

The oral acute toxicity of crude extract of A. pavarii was investigated using astandard protocol for acute oral toxicity[16]. Different doses of crude extract, i.e. 5%, 10%, 15%, 20%, 25%, and 30% (w/v) in gum acacia (5%) were administered orally to various groups of either sex of experimental animals(n=6). Gum acacia suspension (5%) was given for the control. Each group was examined for 24h for any signs of toxicity or mortality, followed by further daily observation for 2 weeks for signs of tremor, Analgesic activity

Hot plate method

The hot plate test was used as a thermal pain model to determine the central antinociceptive activity of the extract. The test was based on the procedure described by Eddy and Leimbach,1953. A transparent glass cylinder was used to keep the animal on the heated aluminium surface.Rats were placed individually on the hot plate and the temperature was kept at 55 ± 0.5°C for a maximum time of 30s to minimize paw damage. The reaction time was recorded when the animal licked its fore and hind paw or jumped off to avoid the heated surface[17].

Acetic acid-induced writhing test

This test was performed to determine the peripheral analgesic activity of the extract.glacial acetic acid was used to induce chemical nociception. Mice were fasted overnight with free water access.Two hours after the mice were given the vehicle orextract, acetic acid 3% (1ml/100gm) was injected (IP) to all mice. After 5 min of acetic acid

administration,the number of writhes produced was counted for 30 min for each Anti-inflammatory activity

Formalin-induced paw oedema test

In the formalin test, the anti-inflammatory effect of A. pavarii extract was studied in male albino rats by injecting 0.1 ml of 2% of formalin in the subplanter region of the right hind paw.After 1h of vehicle and extract administration, formalin was injected. Paw volume was measured before and 1, 2, 3, 4 and 24 h after injection of formalin by a digital plethysmometer, and the percentage of oedema inhibition was calculated[19].

Carrageenan-induced paw oedema test

The carrageenan test was carried out according to the method of Winter, 1962.Acute inflammation was induced by injecting of carrageenan suspension (1% w/v in normal saline, 0.1mL) into the plantar surface of the right hind paw. One hour after the rats were given the vehicle or extract, carrageenan was injected into all rats. Paw volume was measured with a digital Plethysmographimmediately before and after 1, 2, 3, 4, and 24h of carrageenan injection. The increase in paw volume was compared to the control, and percentage inhibition of oedema was calculated[20].

. Statistical analysis

Descriptive statistical analysis was carried out for the generated data from different tests using SPSS (Software package, version 25). Also, the data were tested to find out whether the observed samples are normally distributed using Kolmogorov-Samirnov-maximum deviation test for goodness of fit. If the parameters were normally distributed, the statistical analysis of results was done by using a t test: paired two sample for means otherwise, groups where compared by using Mann-Whitneytest.All experimental results obtained were represented as mean ± standard deviation of mean (S.D.M) of responses. For all tests, the values were considered significant at a level of $p \leq 0.05$. The analyzed data were then presented using bar charts.

Result

Acute oral toxicity study

The acute toxicity test of the six different doses of A. pavarii extract illustrated thatno mortality within 24 h and there are no signs of toxicity observed in rats

and mice within 2 weeks after administration of the extract. Therefore, APLE is considered to becompletely safe up to 600mg/100g, P.O.

Hot plate test

The resultof thehot platetestis represented in figure 1. APLE at a dose of 30% w/v showed asignificant ($p<0.001$) increase in mean reaction time to thermal stimuliwhen compared tothe control.

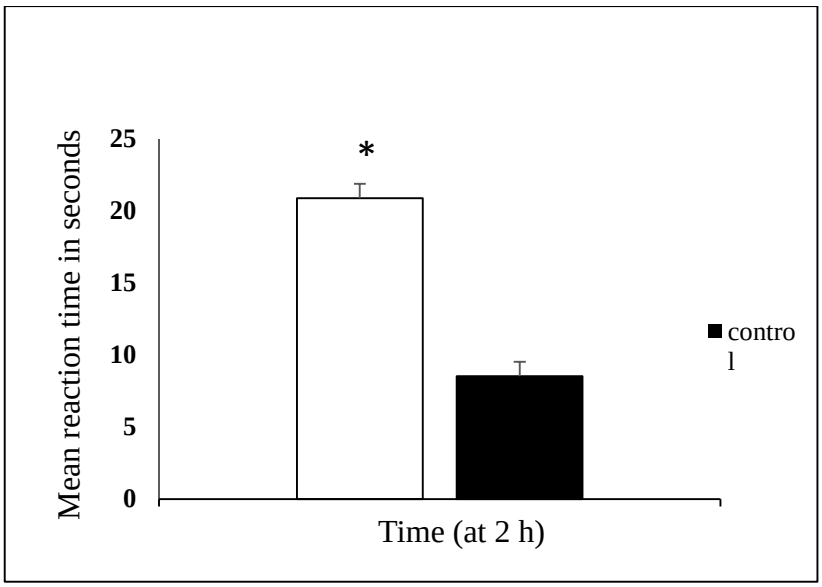

Figure1: The central analgesic activity of 30% APLE by hot plate method in rats. The values represented in the graph are the means (seconds) ± S.D.M. *: *p<0.05*; Significantly different from the control group.

Acetic acid writhing test

The effect of APLE on the writhing response in mice is shown in Figure 2. APLE at a dose of 30% w/vcompletely masked the pain reflex. However, the control exhibited more than 40 writhes ($p<0.0001$). Therefore, the extractattained a 100% inhibition rate of writhing reflex.

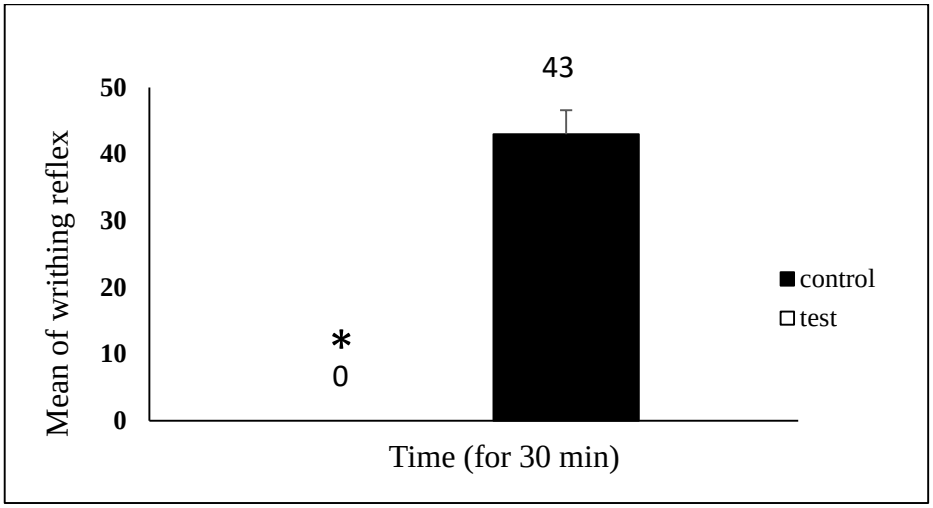

Figure2: The peripheral analgesic activity of 30% APLE by acetic acid-induced writhing reflex in mice. The values represented in the graph are the means ± S.D.M of the writhing number, (n=6). *: *p<0.05*; Significant different from the control group**.**

Formalin-induced paw oedema test

As shown in figure 3, A. pavarii extract at a concentration of (30% w/v) significantly (p<0.001) reduced paw oedema volume in rats. The reduction was detected from the 1sth of formalin injection and continued until the 24th h after extract administration. Markedly, the maximum percent of anti-inflammatory activity was observed at the 1st and 24thh withpercentage valuesof 30.10% and 32.39% respectively.

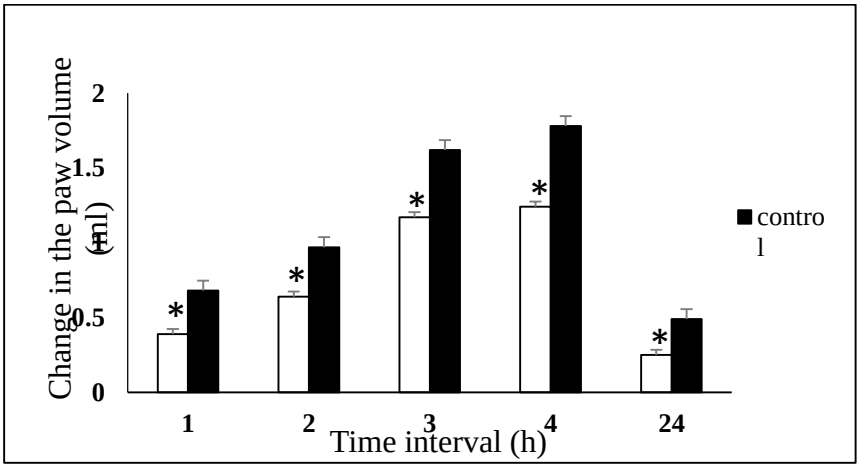

Figure3: The anti-inflammatory activity of 30% APLE by formalin-induced paw oedema in rats. The values represented in the graph are the means (ml) ± S.D.M of paw volume, (n=6). *:*p<0.05*;Significantly different from the control group.

Carrageenan-induced paw oedema test

The data of carrageenan-induced paw oedema is presented in figure4. It wasclearly observed that APLE (30%w/v) produceda significant reduction in the volume of paw oedema resulted from carrageenan injection compared to the vehicle control group ($p<0.001$). Remarkably, the extract administration reduced the rat paw volumeby 30.67%.

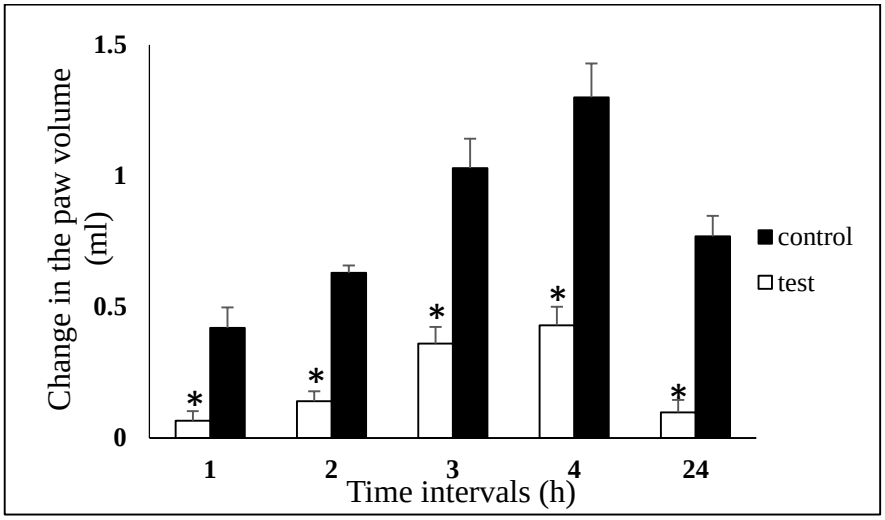

Figure4: The anti-inflammatory activity of APLE by carrageenan-induced paw oedema in rats. The values represented in the graph are the means (ml) ± S.D.Mof paw volume, (n=6). *:$p<0.05$,Significantly different from the control group.

Discussion

Arbutus pavarii leaves and barks have been traditionally used by Libyan people as a remedy for gastritis, renal infections, cold, tuberculosis and various other illnesses[6]. The existing phytochemical data shows the presence of many bioactive compounds such as phenols, flavonoids, carbohydrates, glycosides, unsaturated sterols, triterpenes, and tannins [12].

In this study, the analgesic activity of APLE was investigated as the Irwin test pointed tothe possibility of the presence of ananalgesic effect. Therefore, the analgesic and also anti-inflammatory activity was studied, as occasionally there is a correlation between the two effects[21]. Analgesic drugs are indicated for pain management, and their activities appear to be mediated through peripheral components (aspirin like action) and/orcentral mechanisms (morphine like action). Hence, theanalgesic activity of the extract was

assessed centrally using the hot plate method and also peripherally using glacial acetic acid chemically induced pain test. The results of the hot plate test demonstrated that the oral administration of APLE significantly prolongedthe mean latency time as compared to the control group (figure 1). Furthermore, the extract at a 30% dose produced a completeinhibition of the writhing response induced by acetic acid in mice (figure 2). Therefore, the anti-nociceptiveactivity exerted by APLE probably is mediated by modulating both the central and peripheral pathways of pain. Notably, triterpenes which is one of the various constituents present in Arbutus pavarii was shown to have strong analgesic and anti-inflammatory activity[22],[23],[24]. Therefore, the analgesic and anti-inflammatory effects could be contributed totriterpenes and/or other unrevealed compounds present in APLE. Based on generated data, some active compound(s) in A. pavarii plant can exert peripheral antinociceptive activity, as well as central analgesic activity that may be related tothe stimulation ofopioid receptors.However, more studies are required to elucidate the exact mechanism of action.

Moreover,ethanoic extract obtained from A. pavarii leaf exhibited significant anti-inflammatory activity. The extract suppressed the paw oedema induced by ether formalin or carrageenan in rats.Flavonoids, and phenolic compounds are well known to produce good anti-inflammatory effects[25],[26].One possible mechanism for the anti-inflammatory properties of APLE is the action of flavonoids, triterpenes and phenols that is present in the extract[12]. Remarkably,flavonoids and triterpenes inhibit the prostaglandin synthetase enzyme (cyclooxygenase) that produces prostanoids which mediate the process of inflammation[27]. In addition, prostaglandins are the main player in generating pain, and therefore their inhibition may be contributed to the analgesic activity of the extract.

CONCLUSION

Our findings suggest that the ethanolic extract of leaves of A. pavarii may contain bioactive compound(s) that have strong analgesic and anti-inflammatory activities. However, further studies are recommended to investigate the exact

mechanism of action of APLE and its involvement in pain and inflammatory inhibitory mechanisms.

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