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Evaluation of Analgesic Activity of the Methanol Extract from the Leaves of *Arum palaestinum* in Mice and Rats

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ABSTRACT

Arum palaestinum has a wide use in traditional medicine. It used in folk medicine as a remedy for treatment of post-delivery pain, inflammation, and internal infections. The analgesic activity of the methanolic extract of *Arum palaestinum* was evaluated using various experimental models in mice and rats. Antinociceptive effect of methanolic extract of *Arum palaestinum* was carried out both peripheral and central models. The possible role of opioid receptors in analgesic activity of methanolic extract of *Arum palaestinum* was also examined in hot plate test using naloxone. Methanolic extract of *Arum palaestinum* at all three test doses (5, 10 and 20 mg/kg body weight) significantly ($p < 0.05$) decreased the pain response in all nociceptive models in dose-dependent fashion. Pretreatment with naloxone (2 mg/kg) caused significant ($P < 0.05$) change in the analgesic activity of 20 mg of methanolic extract of *Arum palaestinum*. In conclusion, the antinociceptive effect of methanolic extract of *Arum palaestinum* involves activation of peripheral (partly related to lipoxygenases and/or cyclo-oxygenases) and central mechanism (partly, via activation of opioid receptors).

Keywords: *Arum palaestinum*; Analgesic activity; Hot-plate test; Formalin test; Analgesy-meter; Randall–Selitto tests; Peripherally mediated; Centrally mediated; Rats, Mice.

INTRODUCTION

Arum palaestinum Boiss (Araceae), popularly known in our region as "Al-Louf", is a perennial flowering plant native to levant and other Mediterranean region[1]. The Arum genus comprises about 26 species, *Arum palaestinum* is one of these species[1]. *Arum palaestinum* has a wide use in traditional medicine. It ingested as herbal remedy to treat post-delivery pain, inflammation, and internal infections[2, 3]. The aerial parts of *Arum palaestinum* are cut up in Jordan and thoroughly cooked with olive oil and lemon after being drying because it is perceived to protect from cancer (recommended by the traditional healers)[2]. The plant is also used as animal feed resources. Few studies have demonstrated that *Arum palaestinum* has phytochemical and biological activities. Of theses, Afifi *et al*., (1999)[4] showed that *Arum palaestinum* extract reduces muscle contraction of uterus in rat and guinea pig. Further, *Arum palaestinum* has been shown to have anti-proliferation and antioxidant effect[5, 6]. Phytochemical screening of the *Arum palaestinum* demonstrated the presence of (S)-3,4,5-trihydroxy-1 H-pyrrol-2(5H)-one alkaloid, caffeic acid, isoorientin, luteolin, vicenin 11, 3,6,8-trimethoxy, 5,7,3',4'-tetrahydroxy flavone, vitexin (apigenin 8-C-glycoside), isoorientin (luteolin 6-C glucoside), chrysriol, and chryseriol-7-glucoside[5, 4].

MATERIALS AND METHODS

Preparation of plant material and extracts

Plant material

In this study, the plant part utilized was the leaves. The fresh leaves of *Arum palaestinum*

were obtained from Ajloun (Jordan) between the months of March and May. The plant material was identified and authenticated at the Herbarium section of the Department of biological sciencs, Hashemite University. A voucher specimen was deposited at the Hashemite University Herbarium for future reference.

Preparation of methanol extract

The fine powder leaves was weighed (1000g) and soacked in 2500 ml of methanol for 36 h with constant shaking. The mixture was clarified by filtration using Whatman's filter paper and the resultant filtrate was concentrated under low tempreture (40°C) and reduced pressure to gummy materials in rotary evaporator. The final yield was 5.92% (w/w) of the extract.

Phytochemical analysis

Phytochemical analysis of the methanol extract of the plant was based on a standard procedure[7, 8]. The methanol extract was tested for alkaloids, tannins, saponins, glycocides and saponin glycosides. Thin layer chromatography was used to confirm the presence of these constituents.

Animals

Male and female Wistar rats weighing 180–250 g and Swiss albino mice weighing 18–25 g were used in this study. The experimental animals were provided the Animal House of Biology Department, Hashrmite University. Animals were maintained under ambient conditions. Rooms temperature was maintained at 22±2 æ%C Alternate 12 hr light/ dark cycle was also maintained. Food and water were provided *ad libitum* to all animals. Prior to commencement of the experiments, the animals were fasted for Twelve hours but still had access to water. Animal care and experimental protocols were performed in accordance with NIH publication No: 85-23, revised in and approved by the Animal Care Committee at the Hashemite University.

Acute toxicity studies: LD50

The determination of the median (LD50) of the plant extract was perform according to the methods described by Lorke (1983) with some modification[9]. Sixty mice were fasted overnight and then were divided into two sets containing 6 male and female mice in each set. Methanol plant extract was administrated to set I orally at the doses of 10,100 and 1000 mg/kg in the first stage of determination while set II was treated intraperitoneally (i.p.) at same doses. The mice were allowed food and water *ad libitum* and observed for 24 h after treatment for signs of acute toxicity or death. The results of this stage help us to select the doses for the next stage. 25, 50 and 100 mg/kg (p.o.) and 200, 400 and 600 mg/kg (i.p.) were the selected doses for the next stage. The final median lethal dose (LD50) value was estimated as the geometric mean of the highest non-lethal dose and the lowest lethal dose in the second stage.

Acetic-acid induced abdominal writhing test

The writhing test was based on the method described by Koster *et al.* (1959)[10] with modifications in timing of observations. Animals injected with acetic acid (6%, 10 mL/kg) intraperitoneally to induce abdominal writhing. A total of 30 mice were divided into 5 groups (n = 6 per group) and were pretreated 30 min prior to acetic acid injection as follows: groups I, II and III: injected intraperitoneally with 5, 10 and 20 mg/kg of the extract, respectively; group IV: injected intraperitoneally with acetylsalicylic acid (ASA) 150 mg/kg (as positive control) and group V: injected intraperitoneally with distilled water (vehicle control). The number of writhings was counted twice (at 15 min intervals)over a period of 5 min after a 5-min lag period after the injection of acetic acid and expressed as % of constriction inhibition. The assessment was performed in a blind fashion.

Formalin test in rats

The test was conducted according the method of Dubuisson and Dennis (1977)[11] with slight modification. Briefly, rats were divided into six groups (six rats each) and were trated intraperitoneally with either distilled water (control), 5, 10 and 20 mg/kg extract, ASA (150 mg/kg) and morphine (4 mg/kg). 30 min later, animals were given an intraplanter injection of 50 ¼L of formalin (2.5%) into the right paw using 27-gauge needle. Immediately post formalin administration animals were placed individually in acrylic observation chambers (320 $cm^2 \times 40\ cm^2$) and monitored for 1 h. The nociceptive responses was recorded over a period of 40 min with 10min lag period (early phase, 0-5 and late phase 15-40min) and assessed using the following 4 points scoring system: (0) rats and stand or walked firmly on injected paw; (1) the injected paw was not fully lifted; (2) the injected paw was completely lifted off

the floor; (3) the rat licked or chewed the injected paw.

Hot-plate test in mice

This test was carried out according the method of Turner (1965)[12]. The mice were divided into 5 groups (n=6 per group) and treated as following: groups I, II and III: injected intraperitoneally with 5, 10 and 20 mg/kg of the extract, respectively; group IV: injected intraperitoneally with morphine (4mg/kg, as positive control) and group V: injected intraperitoneally with distilled water (vehicle control). Animals were then placed on hotplate (UGO Basile, Italy) kept at a temperature of 53 ± 0.5 æ%C. The reaction time was then observed over the period of

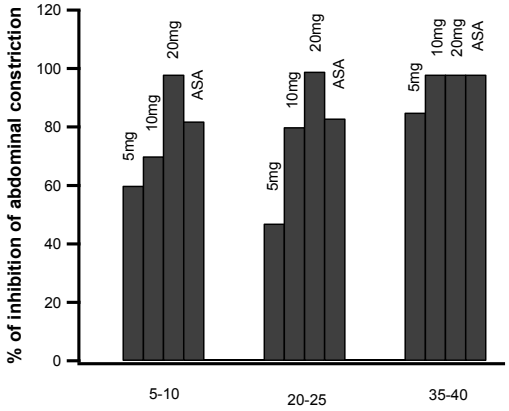

Fig. 1: Antinociceptive activity of methanolic extract of the leaves of *Arum palaestinum* and ASA 150 mg/kg in acetic acid induced writhing test. Control values indicate the animals injected with distilled water. *(P* < 0.05, *n* = 6)

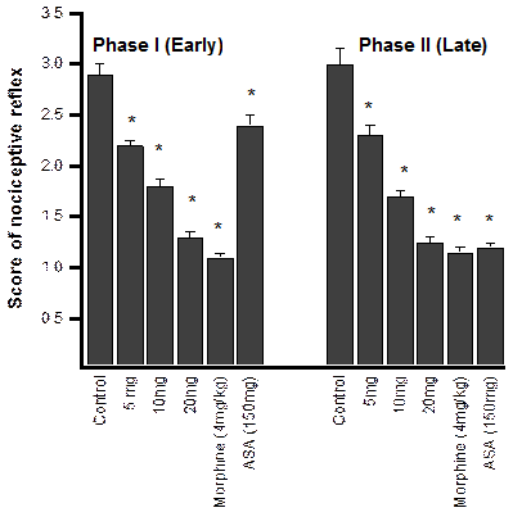

Fig. 2: Effects of the methanolic extract of the leaves of *Arum palaestinum* and ASA 150 mg/kg on formalin test. Control values indicate the rats injected with distilled water . * *P* < 0.05 compared with control group (*n* = 6)

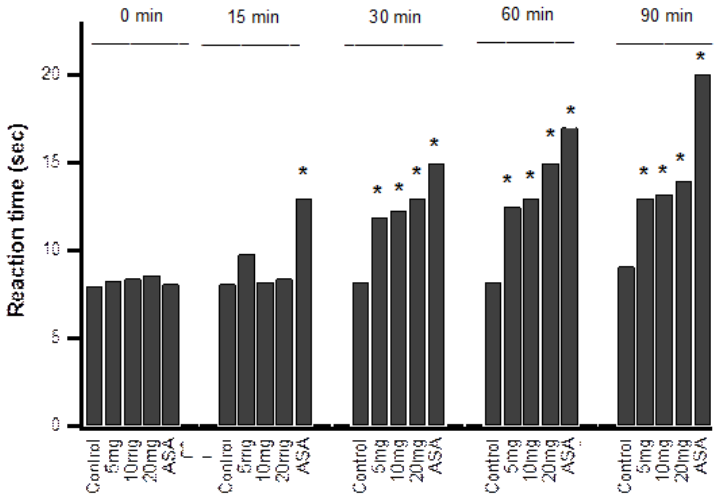

Fig. 3: Antinociceptive activity of the methanolic extract of the leaves of *Arum palaestinum* and ASA 150 mg/kg in hot plate reaction time. Control values indicate the animals injected with distilled water. * *P* < 0.05 compared with control group (*n* = 6)

90min (0, 15, 30, 60 and 90 min) after extract or drug administration. The reaction time was considered as the time elapsed between placing of the mouse on the hotplate and licking of the hind paw or jumping. The increase in reaction time was determined for each extract- and drug-treated group and is expressed as percentage.

Analgesy-meter induced pain

Analgesy-meter test was carried out in rats using an Ugo Basile analgesy-meter (model 7200, Italy). A constantly increasing force was applied to the rat paw by the plunger. Pain was determined by the physical struggles of the animal to free its paw. Rats were used in groups of six per dose of plant extract (groups I, II and III received 5, 10 and 20 mg/kg, i.p.), distilled water (group IV i.p.), or morphine (4 mg/kg, group V (s.c.)). Readings were taken at intervals of 15 minutes for 30 minutes (0, 15 and 30 min) after drug and extract administration. The assessment was performed in a blind fashion. The increase in pain threshold for each extract- or drug-treated group was compared with that of contrl group and was expressed as percentage.

Randall–Selitto test

The method of Randall and Selitto (1957)[13] was used in this test. Rats were divided into 6 groups. Rats in group 1,2,3 were injected with 5,10,20mg/kg methanol extract, respectively. Rats in group 4, 5, 6 were received distilled water morphine (4 mg/kg) and ASA (150 mg/kg). 30 min later rats were received 50 µl of raw egg albumin into the subplanter surface of rat left hind paw to induce edema. The threshold pain (the force (g) applied to the dorsal surface that caused animal to withdraw the paw) to mechanical stimulus was determined for both inflamed and intact paws of each group by means of analgesiometer (model 7200). The increases in the nociceptive response for both inflamed and intact paws in comparison to control measurements were calculated and was expressed as percentage.

Evaluation of the mechanism of antinociceptive action

To evaluate the mechanism of action of the methanolic extract of *Arum palaestinum* on pain. Animals were pre-treated with the opioidergic receptor antagonist naloxone (2 mg/kg). Naloxone was administrated intraperitoneally 10 min before administration of distilled water, plant extract (20 mg/kg) or morphine (4 mg/kg). Hot-plate test was performed as mentioned above. The reaction time

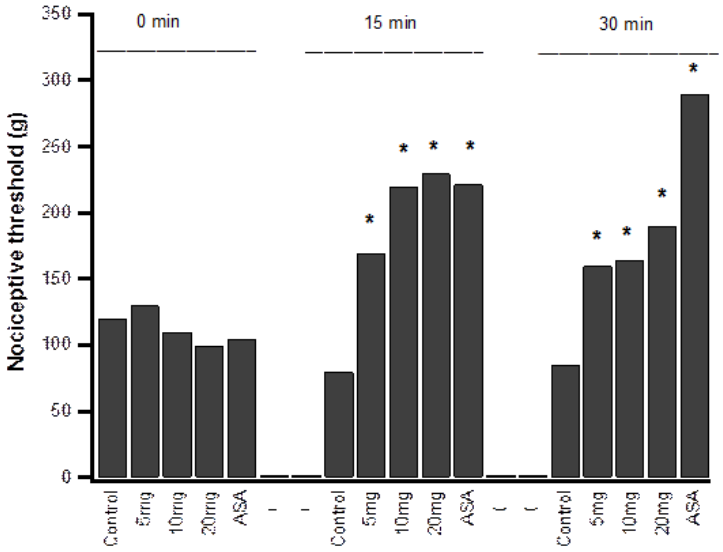

Fig. 4: Antinociceptive activity of the methanolic extract of the leaves of *Arum palaestinum* and ASA 150 mg/kg in analgesy-meter test. Control values indicate the animals injected with distilled water . * $P < 0.05$ compared with control group ($n = 6$)

was recorded over the period of 90 min after extract and drug administration.

Statistical analysis

The data obtained were presented as Mean ± Standard Error Mean (SEM) and analysed using two-way ANOVA followed by Student's *t*-test. Microsoft Excel®and STATISTICA® computer software were also used for data analysis. $P < 0.05$ indicates statistical significance

RESULTS

Phytochemical screening

Data obtained from the phytochemical analysis of *Arum palaestinum* indicated the presence of the all secondary metabolites tested.

Acute toxicity studies

The acute toxicity study shows a decreased mobility, respiratory distress (gasping) with eventual immobility but no convulsions or loss of righting reflex prior to death in the animals. The calculated LD_{50} of the methanolic extract of *Arum palaestinum* was 94.3±5.5 mg/kg i.p. and 735.1±23.5 mg/kg p.o. in mice.

Antinociceptive studies

In this study, i.p. injection of methanolic extract of *Arum palaestinum* showed a dose-dependent antinociceptive activity in all nociception models used (Figs. 1–4 and Table 1).

Acetic-acid writhing test

The administration of methanolic extract of *Arum palaestinum* at 5,10, and 20mg/kg had significant ($P < 0.05$) inhibitory effect on the number of acetic acid-induced abdominal constrictions in mice as compared with control. The effect was dose-dependent and increase with time. The inhibitory effect was (93.3%) at 20mg/kg over the first 5–10 min of monitoring (Fig. 1). The reference drug ASA also significantly ($P < 0.05$) inhibited the abdominal constrictions compared to control.

Formalin test in rats

As shown in Fig. 2 the methanolic extract of *Arum palaestinum* had significant ($P < 0.05$) antinociceptive activity in the formalin test. The extract reduced significantly ($P < 0.05$) both the early (neurogenic, 0– 5 min) and late (inflammatory, 15–30 min) phases of the formalin-induced nociception method at 5, 10, and 20 mg/kg. In the early phase, the maximal percentage of inhibition of nociceptive response was 53.3% at a dose of 20 mg/kg. Morphine (4 mg/kg), used as reference drug, produced significant ($P < 0.05$) and equal reduction in the paw licking of both phases compared to control. ASA (150 mg/kg) significantly ($P < 0.05$) produced more inhibition of licking in the late phase than early phase.

Hot-plate test in mice

The pretreatment of animals with the methanolic extract of *Arum palaestinum* (5–20 mg/

Table 1: Effects of the methanolic extract of the leaves of *Arum palaestinum*, ASA (150 mg/kg) and morphine (4 mg/kg) on albumin-induced edema in rats (Randall–Selitto test).

Treatment group (dose mg/kg)	Pain threshold (g)/time (min)					
	0	30	60			
	Intact	Inflammed	Intact	Inflammed	Intact	Inflammed
Control	176.1 ± 23.3	162.1 ± 31.1	161.5 ± 32.3	150.5 ± 22.1	168.0 ± 22.4	152.2 ± 19.7
Extract (5)	154.2 ± 34.3	147.2 ± 30.4	241.4 ± 37.1*	219.4 ± 20.2*	248.1 ± 15.3*	244.3 ± 17.9*
Extract (10)	155.6 ± 25.1	146.6 ± 27.7	278.2 ± 35.9*	225.2 ± 15.4*	278.8 ± 17.1*	282.3 ± 34.1*
Extract (20)	156.1 ± 21.8	138.8 ± 33.9	290.9 ± 44.7*	238.3 ± 27.9*	282.4 ± 37.6*	238.5 ± 39.3*
Morphine (4)	149.7 ± 19.5	136.3 ± 22.5	250.6 ± 23.5*	378.8 ± 37.6*	292.2 ± 39.9*	364.7 ± 57.28
ASA (150)	151.3 ± 19.9	144.2 ± 26.8	210.4 ± 21.6*	383.7 ± 57.5*	231.3 ± 20.8*	362.1 ± 33.6*

Control values indicate the animals injected with distilled water.* $P < 0.05$ compared with control group. Values are expressed as mean ± S.E.M. ($n = 6$)

kg) resulted in a significant dose-dependent increase in the reaction time to thermal stimulation compared with control group (Fig. 3). The highest increase in reaction time was observed with the dose of 20 at 60-min post-treatment (69.8%). The percentage of increase in reaction time produced by the standard drug morphine (4 mg/kg) was 105.3% at 60 min.

Analgesy-meter induced pain study

Fig. 4 shows that the pretreatment with methanolic extract of *Arum palaestinum* (5–20 mg/kg) evoked a significant dose-dependent and time related ($P < 0.05$) antinociceptive effect when the extract compared to control group (Fig. 4). The maximal percentage increases in threshold of nociceptive reflex was 60.1, 109.7, and 120.7%, respectively, all at 15 min. The morphine (4 mg/kg), standard reference drug, produced significant ($P < 0.05$) effects compared to control group.

Randall–Selitto test

In the Randall Selitto test, the methanol extract of *Arum palaestinum* (5–20 mg/kg) significantly ($P < 0.05$) and in dose dependent manner increase the pain threshold in the inflamed and intact paws in comparision with control group over thirty minutes period (Table 1).

Evaluation of the mechanism of antinociceptive action

Pretreatment of animals with antagonists naloxone significantly ($P < 0.05$) reversed the antinociceptive action of methanolic extract of *Arum palaestinum* (20 mg/kg) and morphine (4 mg/kg). The methanol extract dose used was chosen on the basis of hot plate test results (the dose that gives maximal response)

Discussion

In preliminary experiments it was found that the methanolic extract of *Arum palaestinum* was more active and easier to maintained than the aqueous extract and thus it was selected for this study. Furthermore, using methanol as a solvent may maximize the extraction of some polar solutes pharmacological investigations[14].

The antinociceptive effect of the methanolic extract of *Arum palaestinum* has been investigated. The results of the present study showed that the methanolic extract elicited potent antinociceptive activities that assessed using different pain models. The present results also demonstrated that the methanolic extract of *Arum palaestinum* act both centrally and peripherally. The pain models used in this study were selected such that both centrally and peripherally mediated effects were measurable[15]. Non-selective opioid receptor antagonist was used to anticipate the mechanism involved in methanolic extract of *Arum palaestinum* antinociceptive properties.

The writhing test is a chemical induced nociception indicating involvement of both central and peripheral mechanisms. In the writhing test, acetic acid injection activates the synthesis of prostaglandins which induce abdominal constrictions due to activation of peripheral nociceptors[16]. Non-steroidal anti-inflammatory drugs like aspirin reduce the level of prostaglandin which reduce the sensitivity of the nociceptors to pain inducing agents such as bradykinin[17-20]. Administration of methanolic extract of *Arum palaestinum* produced a significant dose-dependent decrease in the number of acetic acid-induced writhes. This observation points out that methanolic extract of *Arum palaestinum* possess peripherally-mediated antinociceptive property that may work via reducing the level of prostaglandin synthesis or other inflammatory mediators.

Formalin test produces a biphasic pain response: the first phase (0-15min) is supposed to be reflective of neurogenic pain (direct activation of C- fiber afferent nociceptors) while the second phase (15-30min) is supposed to be due to the release of inflammatory mediators[23-25]. In formalin test, centrally acting analgesics reduce both phases almost equally[15] whereas peripherally acting analgesics only reduce the late phase[21-22]. Considering the inhibitory property of the methanolic extract of *Arum palaestinum* on both phases of pain response relative to controls, one can suggest that the methanolic extract of *Arum palaestinum* has both central and peripheral antinociceptive activities. This suggestion is in line with the significant effect seen in the hot-plate and acetic acid-induced abdominal writhing tests.

The the hot-plate test and analgesy-meter test has been used for evaluation of centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level. The dose dependent antinociceptive effect seen in the hot-plate test and analgesy meter test clearly indicates the central antinociceptive effect of the methanolic extract of *Arum palaestinum.*

Randall–Selitto test is used to measure analgesic activity based on the fact that inflammation increases pain sensitivity by reducing the pain threshold and this sensitivity is modifiable by analgesics[13, 15]. Peripherally acting analgesics, such as NSAIDs, increase only the threshold of the inflamed paw, whereas centrally acting analgesics, such as opiate, increase the threshold of both the inflamed and intact paws. In Randall-Selitto test, the methanolic extract was observed to increase the pain threshold in both the intact and inflamed paws. This result suggests the methanolic extract of *Arum palaestinum* acts via both central and peripheral mechanisms.

The central analgesic action of the methanolic extract of *Arum palaestinum* was confirmed using naloxone, a non-selective opioid antagonist receptors. Naloxone can block spinal and supraspinal opioid receptors in the central nervous system. Pretreatment with naloxone significantly (P<0.05) reverse the antinociceptive action of the methanolic extract of *Arum palaestinum*. This observation suggests that the antnociception induced by methanolic extract is associated with its interaction with opioid system.

In conclusion, the present findings indicate that the methanolic extract of *Arum palaestinum* has antinociceptive effect that involve central and peripheral pathways. The centrally mediated effect may be linked partly to activation opioid receptors, while peripherally effect may be due to the activation of lipoxygenase and /or cyclooxygenase. The effects of methanolic extract of *Arum palaestinum* on pain, explain the uses of lend *Arum palaestinum* in folk medicine in pain control.

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