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ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF AN EXTRACT AND FLAVONOIDS FROM *ARTEMISIA HERBA-ALBA* AND THEIR MECHANISMS OF ACTION

Artemisia herba-alba (*A. h.-a.*) has wide use in traditional medicine for the relief of coughing, healing external wounds, and treatment of pain associated with gastrointestinal disturbances. We investigated *in vivo* antinociceptive and anti-inflammatory activities of an aqueous extract (aq. ex.) and two isolated compounds obtained from aerial parts of *A. h.-a*. The analgesic effects of aq. ex. (10, 31.6, 100, 316, and 1000 mg/kg), astragalin, and eupatilin (both, 0.316, 1, 3.16, 10, 31.6, and 100 mg/kg) were studied using the hot-plate test in mice and formalin test in rats. The effects were compared with those of 5 mg/kg morphine. Dose-dependent analgesic effects of aq. ex., astragalin, and eupatilin were clearly manifested in both hot-plate assay and early and late phases of formalin-induced paw licking. These effects were significantly but partly reduced by the opioid receptor antagonist naloxone (5 mg/kg). The same range of doses of aq. ex., astragalin, and eupatilin caused dose-dependent suppression of carrageenan-induced paw edema in rats. Thus, we demonstrated that *A. h.-a.* possesses noticeable antinociceptive and anti-inflammatory activities; our data support the reasons for using this plant as a remedy for treatment of pain and inflammation. Antinociceptive and anti-inflammatory actions of *A. h.-a.* are considerably related to the presence of astragalin and eupatilin.

INTRODUCTION

Artemisia herba-alba (*A. h.-a.*) is a subspecies of *Artemisia alba (*family *Asteriacea*); sometimes it is qualified as a separate species. It is a strongly aromatic herb, with many erect and leafy stems (20-40 cm in height) and leafs covered by woolly hairs. *Artemisia herba-alba* grows widely in Jordan and in the entire Middle East, as well as in some other regions of Europe and Asia. In Jordan, this plant is common in dry mountains and desert regions [1].

Artemisia herba-alba has wide use (mainly as anthelmintic and antispasmodic) in traditional medicine. It is also used as a remedy for coughing, external wounds, falling hair, gastric disturbances, and jaundice [2-6]. It was also reported that a tincture of the leaves of this species can be used as an antidiabetic (hypoglycemic) and sedative drug [7]. Moreover, there was reported that agents from this plant manifest antifungal, antibacterial, leishmanicidal, antipyretic, antiallelopathic, and antioxidant activities [8, 9]. Recently, the neurological activities of an ethanolic extract and some flavonoids from *A. h.-a.* have been demonstrated [10]. An aqueous extract (aq. ex.) of A. *h.-a.* was found to inhibit hemolytic activities of the viper and scorpion venoms [11].

Phytochemical studies of *A. h.-a.* revealed the presence of santonin, sequiterpene lactones, camphor, 1,8-cineole, *p*-cymene, davanone, cineol-thujanebornane, pinane, α- and β-thujones, a chrysanthenyl derivative, quercetin-3'-glucoside, quercetin-3-O-rutinoside, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, dinatin (4',5,7-trihydroxy-6-methoxyflavone), skrofulein (4',7-dihydroxy-6,7'-dimethoxyflavone), essential oils, and eudesmanolides [2, 6, 12-15]. Components of the essential oil from A. *h.-a.* have also been investigated [16-19].

Despite the traditional use of A. *h.-a.* as an analgesic, no systemic studies concerning its

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antinociceptive effects have been carried out to affirm the traditional use and to establish chemical principles responsible for the activities reported. Here, we describe for the first time the antinociceptive and anti-inflammatory effects of an aqueous extract (aq. ex.) of *A. h.-a.* aerial parts and two known flavonoids (astragalin and eupatilin) isolated from this plant. In addition, our results showed that these two flavonoids are the major compounds of this plant responsible for its antinociceptive effects.

METHODS

Plant Material. Aerial parts of wild-growing A. *h.-a.* were collected during April from Al-Mafraq (Jordan) by one of the authors. The plant material was identified and authenticated taxonomically at the Hashemite University herbarium. A voucher specimen was deposited under the number HU-255 at the Hashemite University herbarium, Zarka, Jordan, for future reference.

Preparation of the aq. ex. The extract was obtained by boiling 150 g of air-dried aerial parts of A. *h.-a.* in 1000 ml of distilled water for 60 min with continuous stirring. The resultant solution was filtered through Whatman filter paper. The filtrate was completely evaporated under reduced pressure at 50°C to give 10 g of a gummy material. Solutions were prepared by dissolving weighed amounts of this material in physiological salt solution (PSS). The latter had the following composition (mM): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; $NaH_2PO_4 \cdot H_2O$, 1; Na_2HPO_4, 0.5; glucose, 11.1; $MgCl_2 \cdot 6H_2O$, 0.5, and $CaCl_2 \cdot 2H_2O$, 2.5; pH of the stock solution was adjusted to 7.4.

Extraction and Fractionation Procedures. Air-dried and powdered aerial parts of *A. h.-a.* (3500 g) were extracted by successive macerations with dichloromethane and 50% aqueous ethanol. Extracts were filtered and concentrated in vacuum to obtain dry dichloromethane (5.3%) and 50% ethanol extracts (16.2%). The dichloromethane extract was adsorbed on Celite and eluted successively with hexane, toluene, diethyl ether, and acetone, with a final wash with methanol. Four main fractions, F1–F4, were obtained and dried under vacuum. The F3 was separated on a Silicagel 60 column (Merck, Germany) eluted with gradients of cyclohexane–ethyl acetate and ethyl acetate–acetone to yield 11 fractions. Fractions 3.V and 3.VI were combined (F 3.V–VI), submitted to gel permeation on a Sephadex LH-20 column (Pharmacia, USA), and eluted with methanol to afford 36 subfractions. Subsequent differentiation of the constituents of these fractions using chromatographic techniques allowed us to obtain the necessary amounts of compounds 1 and 2. The structure of these compounds corresponded to those of astragalin and eupatilin (5,7-dihydroxy-6,3',4'-trimethoxyflavone), respectively (Fig. 1), through comparison of their 1H NMR, MS, and IR with those previously reported in literature [20, 21]. Other extracts and fractions were not investigated in our study since their biological activity was low.

Experimental Procedures. Non-fasting male Wistar rats (150-250 g) and mice (22-30 g), housed at 22-25 °C under a 12 h light/dark cycle and with access to food and water *ad libitum*, were used throughout the experiments.

Hot-Plate Test was assessed using groups of male mice, six animals per group. The hotplate temperature was maintained at 50 ± 1°C. The latency of a discomfort reaction, licking paws (sec), was measured before and

A   *B*
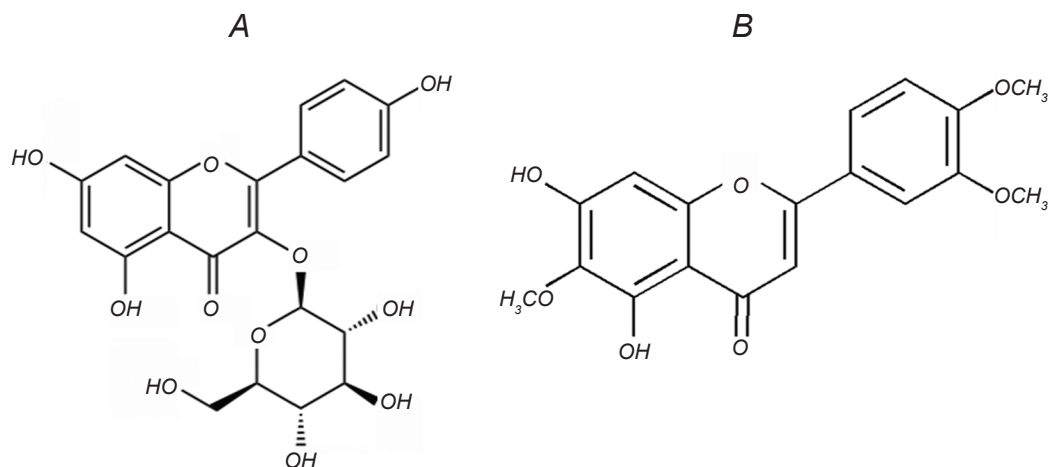
F i g. 1. Chemical structure of astragalin (A) and eupatilin (B).

Р и с. 1. Хімічна структура астрагаліну (*A*) та еупатиліну (*B*).

60 min after i.p. administrations of 10 ml/kg PSS (control), *A. h.-a.* aq. ex. (10, 31.6, 100, 316, and 1000 mg/kg), astragalin, eupatilin (both, 0.316, 1, 3.16, 10, 31.6, and 100 mg/kg), or morphine (5 mg/kg; positive control). The largest doses were determined on the basis of LD50 experiments (for aq. ex.) or values of the practical solubility (for astragalin and eupatilin). Smaller doses were calculated as corresponding to approximately 0.5 log units from each other on the log scale. The cut-off time was 60 sec. Means of three readings of the reaction time obtained before administration of PSS, aq. ex., astragalin, eupatilin, or morphine were considered baselines as normal reaction times of animals to the temperature used. Increases over baseline (%) were calculated according to the mean of three readings of the latency after treatment measured within 5-7 min; the latencies before treatment were taken as 100%. In all experiments, ED50 values were determined from the plot of individual experiments by the best visual fit.

Formalin Test. Rats were divided into groups (six rats each) and injected i.p. with either PSS (control), aq. ex. of *A. h.-a.*, astragalin, or eupatilin in the doses mentioned above, or 5 mg/kg morphine (positive control). Sixty min later, 50 μl of formalin (5%) was injected into the dorsal surface of the right hind paw of each rat using a microsyringe [22]. Immediately after formalin injection, animals were placed individually in acrylic observation chambers (320 cm^2 × 40 cm); clear observation of the paws of the animals was provided by mirrors. Licking of the injected paw was defined as the nociceptive response. Total times of the response were measured during the periods of 0-5 min (early phase) and 15-40 min (late phase); normalized values of inhibition of licking (%) were calculated.

Carrageenan-Induced Edema in Rats [23]. Groups of five animals each were used. Paw swelling was elicited with 0.1 ml 1% carrageenan in 0.9% saline (w/v) injected into the right hind foot under the plantar aponeurosis. Test groups of the rats were treated i.p. 1 h before carrageenan injection with 10 ml/kg PSS (control), aq. ex., astragalin, and eupatilin (in the doses mentioned above), or 10 mg/kg indomethacin (positive control). The inflammation intensity was quantified by measuring the volume displaced by the paw, using a plethysmometer (Ugo Basile, Italy) at time points 0, 1, 2, 3, and 5 h after carrageenan injection. Normalized intensities of inflammation (%) were calculated.

Involvement of the Opioid System in the Antinociceptive Action of *A. h.-a.* To evaluate the mechanism of action of aq. ex., astragalin, or eupatilin, animals were pre-treated i.p. with the opioid antagonist

naloxone (5 mg/kg) 15 min before administrations of PSS or ED50s of the extract, astragalin, eupatilin, or 5 mg/kg morphine. Using the hot-plate and formalin tests as described above, we measured the latencies of nociceptive reactions 60 min after administration.

Statistical Analysis. Numerical values are expressed below as means ± s.e.m. Data were treated by one-way analysis of variance (ANOVA) followed by the Duncan's test for multiple comparisons. Differences were considered significant when $P < 0.05$.

RESULTS

Antinociceptive Effects of *A. h.-a.* Aq. Ex., Astragalin, and Eupatilin.

Hot-Plate Assay. Treatment of animals with the aq. ex. of *A. h.-a.* (10, 31.6, 100, 316, and 1000 mg/kg, i.p., 60 min prior), astragalin, (0.316, 1, 3.16, 10, 31.6, and 100 mg/kg, i.p., 60 min prior), or eupatilin (in the same doses and mode) caused significant dose-dependent ($P < 0.05$) increases in the response latency in the hot-plate test with ED50s of 56.2 ± 3.7, 5.61 ± ± 0.41, and 3.92 ± 0.32 mg/kg, respectively (Fig. 2). The same doses of aq. ex., astragalin, and eupatilin given i.p. did not significantly affect the motor performance of the animals (control response in the rota-rod test was 120 ± 2.1 sec *vs* 120 ± 1.1, 119 ± ± 2.2, and 122 ± 1.6 sec in the presence of the greatest doses of aq. ex., astragalin, and eupatilin, respectively; *n* = 6).

Formalin Test. Intraplantar injections of 5% formalin evoked characteristic biphasic licking responses in the rats. As is shown in Fig. 3, pre-treatment (60 min) with different doses of *A. h.-a.* aq. ex., astragalin, and eupatilin (in the doses mentioned above) caused significant dose-related suppression of both phases of formalin-induced pain in rats. For the early phase, the ED50 values for the aq. ex., astragalin, and eupatilin were 44.5 ± 3.4, 5.6 ± ± 0.31, and 2.26 ± 0.12 mg/kg, while the ED50 values for the late phase were 31.6 ± 3.1, 3.16 ± 0.34, and 1.78 ± ± 0.2 mg/kg for these agents, respectively.

Effects of Naloxone. The results shown in Table 1 indicate that the antinociceptive effect of morphine (5 mg/kg, s.c) was fully reversed by prior treatment of the animals with naloxone (5 mg/kg, i.p.). This was related to both phases of formalin-induced pain and the response latency in the hot-plate test ($P < 0.001$). At the same time, pretreatment of the animals with naloxone in the same dose noticeably but only partly reversed antinociceptive effects of
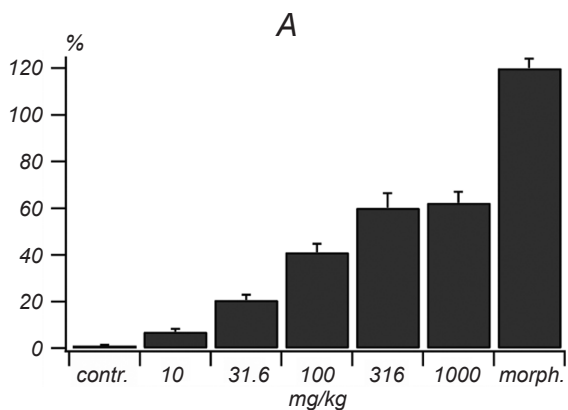
F i g. 2. Effects of the aqueous extract from *Artemisia herba-alba* (A), astragalin (B), and eupatilin (C) on the latency of limb withdrawal in mice submitted to the hotplate test. Values were expressed as means ± s.e.m (*n* = 6). Horizontal scale) Doses of the agents used, mg/kg; contr. is the control, and morph. is morphine, 5 mg/kg. Vertical scale) Normalized increments with respect to the baseline values taken as 100%.

Р и с. 2. Впливи водного екстракту білого полину (*A*), астрагаліну (*B*) та еупатиліну (C) на латентні періоди ноцицептивних реакцій у мишей в тесті „гарячої платівки".
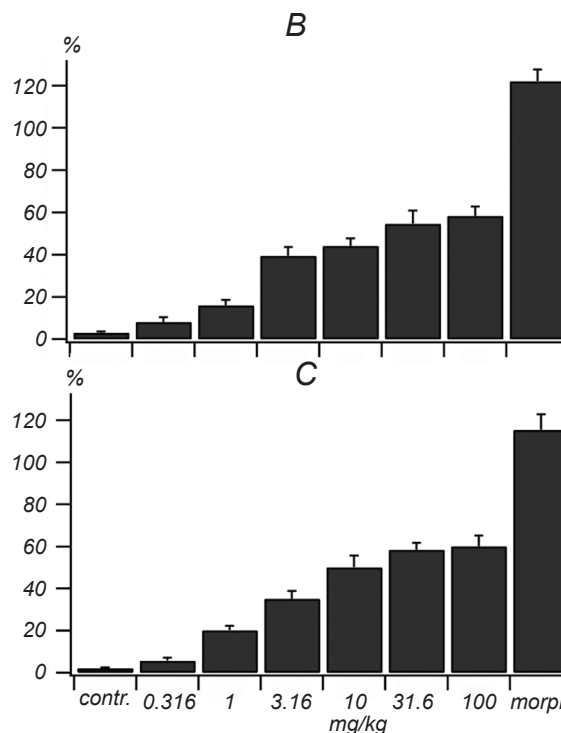
F i g. 3. Effects of the aqueous extract from *Artemisia herba-alba* (A), astragalin (B), and eupatilin (C) on the intensities of the pain reaction (licking the limb) within early (0-5 min) and late (15-40 min) phases of the formalin test in rats. Vertical scale) Normalized values of inhibition, %; the baseline intensities are taken as 100%.

Р и с. 3. Впливи водного екстракту білого полину (*A*), астрагаліну (*B*) та еупатиліну (*C*) на інтенсивність больових реакцій (лизання кінцівки) в перебігу ранньої (0–5 хв) та пізньої (15–40 хв) фаз формалінового тесту у щурів.
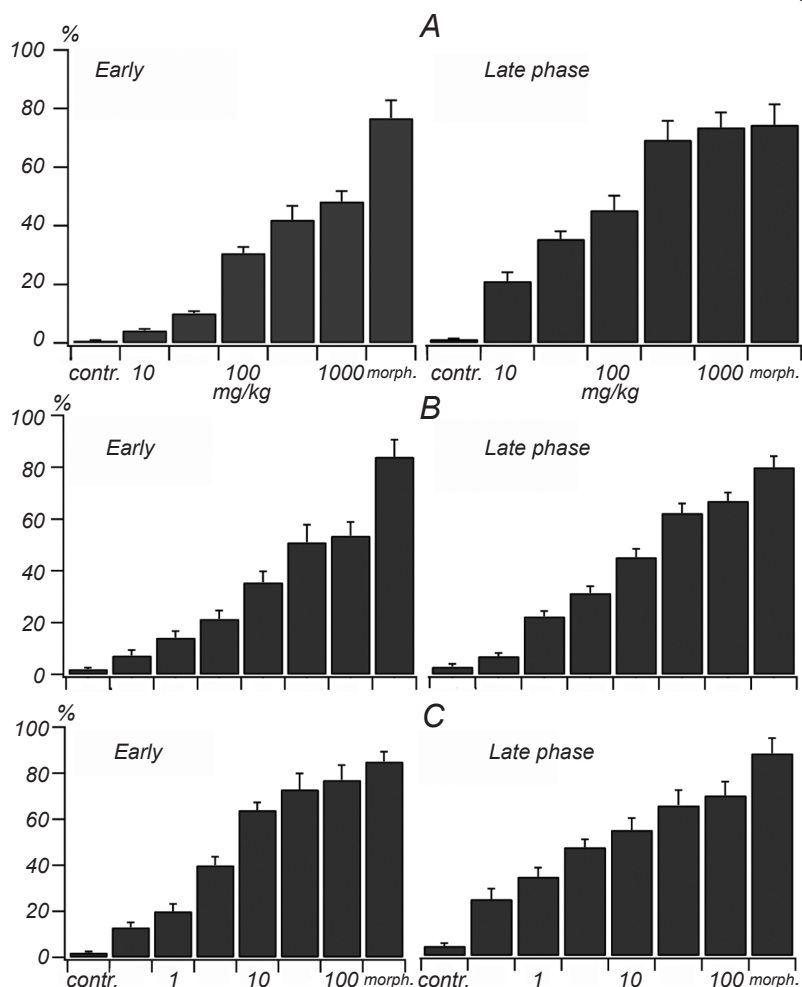
Table 1. Effects of naloxone on the *Artemisia herba-alba* aqueous extract-, astragalin-, and eupatilin-induced antinociceptive activity
Т а б л и ц я 1. Впливи налоксону на антиноцицептивну активність, індуковану екстрактом білого полину, астрагаліном та еупатиліном

Treatment	Hotplate increase in baseline (%)	Inhibition (%) of licking phase I	Inhibition (%) of licking phase II
Control	2 ± 0.30	1.7 ± 0.14	3.1 ± 0.41
Artemisia herba-alba aq. ex. (ED50)	50	50	50
Astragalin(ED50)	50	50	50
Eupatilin(ED50)	50	50	50
Morphine	80 ± 3.70	75 ± 4.10	85 ± 4.52
Naloxone	3.0 ± 0.23	2.7 ± 0.15	1.3 ± 0.10
Morphine+Naloxone	4.1 ± 0.71	5.4 ± 1.20	5.3 ± 0.40
Artemisia herba alba aq. ex. +Naloxone	10 ± 1.10*	15 ± 2.32*	25 ± 3.61*
Astragalin+Naloxone	14 ± 2.10*	25 ± 3.41*	22 ± 1.93*
Eupatilin+Naloxone	18 ± 2.70*	30 ± 5.12*	18 ± 2.70*

Footnotes. Values are expressed as means ± s.e.m. ($n = 6$). *Significant differences from the effects provided by the aqueous extract, astragalin, and eupatilin. The doses of *Artemisia herba-alba* aqueous extract, astragalin, and eupatilin used corresponded to the ED50.

Table 2. Effects of the *Artemisia-herba alba* aqueous extract on carrageenan-induced paw edema in rats
Т а б л и ц я 2. Ефекти водного екстракту білого полину щодо індукованого карагенаном набряку кінцівки у щурів

Treatment	Dose, mg/kg	Time after carrageenan injections, h			
		1	2	3	5
Control		22.3 ± 2.7	34.1 ± 1.4	50.1 ± 2.7	45.2 ± 2.4
Extract	10	21.5 ± 1.6	30.4 ± 1.2*	33.7 ± 1.4*	32.7 ± 1.7*
Extract	31.6	20.2 ± 1.1	27.2 ± 1.1*	28.3 ± 2.1*	27.1 ± 1.3*
Extract	100	22.1 ± 0.9	25.7 ± 1.9*	23.7 ± 1.5*	23.3 ± 2.1*
Extract	316	16.4 ± 1.3*	18.8 ± 1.7*	16.3 ± 0.5*	15.1 ± 1.4*
Extract	1000	14.3 ± 1.2*	11.1 ± 1.6*	10.5 ± 0.7*	13.1 ± 0.7*
Indomethacin	10	10.7 ± 1.5*	9.9 ± 1.3*	10.3 ± 1.2*	11.7 ± 2.5*

Table 3. Effects of astragalin against carrageenan-induced paw edema in rats
Т а б л и ц я 3. Ефекти астрагаліну щодо індукованого карагенаном набряку кінцівки у щурів

Treatment	Dose, mg/kg	Time after carrageenan injections, h			
		1	2	3	5
Control		21.3 ± 1.7	30.5 ± 1.9	52.3 ± 1.6	49.8 ± 3.1
Astragalin	0.316	20.1 ± 1.2	27.3 ± 1.7*	38.3 ± 2.1*	36.3 ± 2.5*
Astragalin	1	22.5 ± 0.9	25.7 ± 1.4*	23.8 ± 1.7*	24.2 ± 1.1*
Astragalin	3.16	21.7 ± 1.6	24.3 ± 1.7*	20.6 ± 2.1*	22.1 ± 3.1*
Astragalin	10	17.5 ± 0.9*	17.3 ± 2.3*	14.2 ± 1.5*	13.2 ± 1.1*
Astragalin	31.6	15.6 ± 1.3*	11.5 ± 1.8*	11.1 ± 1.7*	11.7 ± 1.2*
Astragalin	100	13.1 ± 1.1*	10.6 ± 1.1*	11.3 ± 0.7*	10.2 ± 0.9*
Indomethacin	10	10.4 ± 1.2*	10.1 ± 1.2*	9.8 ± 0.9*	11.3 ± 1.5*

Table 4. Effects of eupatilin against carrageenan-induced paw edema in rats. Values are expressed as means ± s.e.m. ($n = 6$). * Significant differences from the control ($P < 0.05$).
Т а б л и ц я 4. Ефекти еупатиліну щодо індукованого карагенаном набряку кінцівки у щурів

Treatment	Dose, mg/kg	Time after carrageenan injections, h			
		1	2	3	5
Control		22.6 ± 1.3	32.2 ± 3.1	51.2 ± 1.9	47.1 ± 3.5
Eupatilin	0.316	22.3 ± 0.9	29.6 ± 1.2*	35.6 ± 1.5*	33.4 ± 3.2*
Eupatilin	1	19.7 ± 1.2*	26.1 ± 1.1*	26.1 ± 2.4*	23.2 ± 1.5*
Eupatilin	3.16	21.3 ± 1.9*	24.3 ± 1.9*	22.5 ± 1.3*	21.5 ± 2.3*
Eupatilin	10	17.2 ± 1.1*	15.1 ± 1.4*	14.3 ± 0.4*	13.2 ± 0.9*
Eupatilin	31.6	15.1 ± 2.1*	12.2 ± 1.3*	11.2 ± 0.9*	11.1 ± 1.1*
Eupatilin	100	13.3 ± 1.4*	10.5 ± 1.3*	10.3 ± 0.4*	10.1 ± 0.8*
Indomethacin	10	11.2 ± 1.2*	10.1 ± 1.1*	10.5 ± 1.4*	11.3 ± 1.3*

Footnotes. In Tables 2 – 4, values are expressed as means ± s.e.m. ($n = 6$). * Significant differences from the control ($P < 0.05$)

A. h.-a. aq. ex., astragalin, and eupatilin. The effects were more pronounced in the formalin test than in the hot-plate test. Naloxone exerted insignificant effects on the control animals that received PSS. The aq. ex., astragalin, and eupatilin doses used were nearly equal to those corresponding to the ED50s.

Anti-Inflammatory Effects of Aq. Ex. of *A. h.-a.*, Astragalin, and Eupatilin. Preliminary experiments showed that the degree of swelling of the carrageenan-injected rat paws was maximal at 3 h after injection of carrageenan. Tables 2, 3, and 4 show the effects of A. aq. ex., astragalin, eupatilin, and indomethacin in the carrageenan-induced edema test. Statistical analysis proved that inhibition of edema exerted by aq. ex. and its compounds was dose-dependent, and significant differences from the control group were observed. The results showed that astragalin and eupatilin in doses of 100 mg/kg provided anti-inflammatory effects comparable to those produced by 10 mg/kg indomethacin.

DISCUSSION

Our study demonstrated that i.p. administrations of the aq. ex., astragalin, and eupatilin obtained from *A. h.-a.* exerted both clear antinociceptive and anti-inflammatory effects in mice and rats. In the hot-plate test, i.p. treatment by the aq. ex., astragalin, and eupatilin provided antinociceptive effects in a dose-dependent manner. Increases in the reaction latency showing nociception effects in animals treated with the above agents in nearly all doses were significant when compared to control animals treated with PSS. The same delay in the reaction time was obvious when morphine was used in a dose known to have a potent analgesic effect [24]. The efficacies of aq. ex., astragalin, or eupatilin, however, were much lower than that of morphine even when the greatest doses were used (1000, 100, and 100 mg for the aq. ex., astragalin, and eupatilin, respectively). The paw-licking hot-plate response is a complex supraspinally organized behavioral phenomenon [25]. The efficacy of *A. h.-a.* aq. ex. in the hot-plate test might be due to analgesic agent(s) (such as astragalin and eupatilin) acting primarily at the spinal, medullary, and/or higher levels of the CNS or by some indirect mechanisms [26-28].

The results of our study also demonstrated that *A. h.-a.* aq. ex.-, astragalin-, or eupatilin-treated rats showed decreased nociceptive behaviors induced by intraplantar formalin administration during both initial and late phases of the test in a dose-dependent manner. The first acute phase of the nociceptive response in the rat formalin test lasts for about 5 min after formalin injection, and it is followed by the second tonic phase, which persists from 30 to 60 min after injection [26-29]. It is widely accepted that the first phase results from a direct effect on nociceptors activating thin primary afferent fibers (C-fibers), while the second phase represents a tonic inflammatory nociceptive phenomenon [28, 30-31].

The observation that antinociceptive activities of *A.* aq. ex., astragalin, and eupatilin were reduced partly by pretreatment with naloxone, an antagonist of opioid receptors, suggests that the action of the extract, astragalin, and eupatilin may be mediated not only by the action on the above receptors but also by some other mechanisms. Recent studies have shown that formalin activates primary afferent sensory neurons (C-fibers) through a specific direct action on TRPA1s, members of the Transient Receptor Potential family (TRP) possessing cation channels, which are highly expressed in a subset of C-fiber nociceptors [32]. This effect is accompanied by increased influx of Ca^{2+} ions. To investigate the involvement of TRPA1 receptors in the antinociceptive effect of *A. h.-a.* aq. ex., astragalin, and eupatilin, we assessed the cinnamaldehyde-induced nociception test. Currently, it has been demonstrated that intraplantar administration of cinnamaldehyde, a TRPA1 receptor agonist, produces a dose-dependent spontaneous nociception effect in mice [33]. Our results showed that *A. h.-a.* aq. ex., astragalin, and eupatilin significantly suppressed cinnamaldehyde-induced pain (unpublished observations). This result indicates that *A.* aq. ex. constituents (astragalin and eupatilin) probably interact with TRPA1 receptors with C-fibers and reduce the formalin-induced nociception. It has also been proposed that the capsaicin-induced nociception is brought about by activation of another type of TRP receptors, the vanilloid receptors (TRPVs) termed TRPV1; these are ligand-gated non-selective cation channels in primary sensory neurons [34-36]. Our results also showed that administration of *A. h.-a.* aq. ex., astragalin, and eupatilin produced partial (but significant) reduction of the nociceptive responses caused by intraplantar injections of capsaicin into the mouse hindpaw (unpublished observations).

It was shown that CB2 receptor agonists produce antinociceptive effects in models of nociceptive pain [37, 38]. Stimulation of the endocannabinoid release, the influence on cannabinoid-degrading

enzymes, activation of cannabinoid receptors by exogenous cannabinoid-like substances found in *A. h.-a.*, and inhibition of release of chemical mediators of inflammation are other possible mechanisms by which aq. ex., astragalin, and eupatilin from *A. h.-a.* produce antinociception. These possibilities remain only speculative and await further testing.

The second phase of formalin hyperalgesia is based on an inflammatory component since it has been shown that IL-1β and TNF-α are involved in this late phase, and that nonselective COX inhibitors are effective in reducing remote pain in the formalin test [39]. Since aq. ex. of *A. h.-a.*, astragalin, and eupatilin inhibit phase 2 in the formalin test, it is likely that they exert their antinociceptive effects by suppressing the synthesis or production of inflammatory cytokines and mediators such as prostaglandins, histamine, and kinins. This suggestion is supported by the observation that the *A.* aq. ex., astragalin, and eupatilin caused dose-dependent inhibition of carrageenan-induced edema.

Notably, the effects of aq.ex., astragalin, and eupatilin were more prominent during the second tonic phase than within the early phase of formalin-induced pain. This observation suggests that these drugs may be more effective with respect to tonic inflammatory pain when systemically administered.

Carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory effects of *A. h.-a.* aq. ex., astragalin, and eupatilin. Inflammation is characterized by the production of a host of chemical mediators including prostaglandins, leukotrienes, histamine, bradykinins, a platelet activating factor, and cytokines (such as IL-1 and TNF-a), by the release of chemicals from tissues and migrating cells, and also by hyperproduction of reactive oxygen species [40, 41]. Carrageenan-induced inflammation in the paw is a biphasic event [42]. The initial phase observed during the first hour is attributed to release of histamine, serotonin, and cytokines (such as TNF-a), which leads to infiltration of neutrophils and production of neutrophil-derived free radicals (such as hydrogen peroxide, superoxide, and hydroxyl) [43, 44]. The second phase of edema is due to the release of prostaglandins, proteases, and lysosomal enzymes [42, 43]. The observation that aq. ex., astragalin, and eupatilin reduced in the dose-dependent manner carrageenan-induced paw edema suggests that these drugs may inhibit the synthesis and/or production of inflammatory mediators. It is noteworthy that the effects of aq. ex., astragalin, and eupatilin were

nearly the same as that of indomethacin (nonselective inhibitor of COX used as a reference drug) even though the doses used were rather high.

No signs of toxicity, such as diarrhea, motor impairment, ataxia, hyperexcitability, or alterations of the respiratory frequency or piloerection, were noted in the control or experimental animals. Also, no gastric ulcerogenic effect was observed in controls or treated animals. The LD50 for the aq. ex. of *A. h.-a.* was found to be greater than 2000 mg/ kg, indicating the relative safety of this remedy (unpublished observations).

Therefore, the results of our study directly demonstrated, for the first time, that aq. ex. of *A. h.-a.*, astragalin, and eupatilin possess significant antinociceptive and anti-inflammatory properties exerted both centrally and peripherally, as was demonstrated in the hot-plate test, formalin test, and carrageenan-induced paw edema test. These effects seem to be mediated, but only partly, by opioid receptors. Other mechanisms, such as involvement of TRPA1s or TRPVs, have also been discussed.

These results support the ethnomedical use of this plant and suggest that the extract and/or its active constituents might represent potential therapeutic options for the treatment of pain-related diseases.

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АНАЛГЕТИЧНІ ТА ПРОТИЗАПАЛЬНІ ЕФЕКТИ ЕКСТРАКТУ ТА ФЛАВОНОЇДІВ БІЛОГО ПОЛИНУ ТА ЇХ МЕХАНІЗМИ

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Р е з ю м е

Білий полин (*Artemisia herba-alba – A. h.-a.*) знаходить широке застосування в традиційній медицині. Його вживають для припинення кашлю, лікування ран, вгамування болю при шлунково-кишкових розладах та ін. В експериментах *in vivo* ми досліджували антиноцицептивні та протизапальні ефекти водного екстракту (в. екс.) та двох окремих компонентів, отриманих із надземних частин *A. h.-a.* Аналгетичні ефекти в. екс. (10, 31.6, 100, 316 та 1000 мг/кг), астрагаліну

та еупатиліну (обидва в дозах 0.316, 1, 3.16, 10, 31.6 та 100 мг/кг) визначались у тесті «гарячої платівки» на мишах та формаліновому тесті на щурах; ці ефекти порівнювали з такими морфіну. Залежні від дози аналгетичні впливи в. екс., астрагаліну та еупатиліну яскраво проявлялись як у тесті «гарячої платівки», так і в перебігу ранньої та пізньої фаз реакції лизання кінцівки, індукованої ін'єкцією формаліну. Ці ефекти усувались, проте лише частково, антагоністом опіоїдних рецепторів налоксоном (5 мг/кг). Аналогічні дози в. екс., астрагаліну та еупатиліну забезпечували дозозалежне пригнічення індукованого карагеном набряку кінцівки у щурів. Таким чином, ми продемонстрували, що для *A. h.-a.* характерні істотні антиноцицептивна та протизапальна властивості. Наші дані підтверджують обгрунтованість застосування цієї рослини як лікувального засобу в традиційній медицині. Згадані властивості *A. h.-a.* в істотній мірі залежать від наявності в її складі астрагаліну та еупатиліну.

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