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ANTI-NOCICEPTIVE EFFECT OF AGRIMONIA EUPATORIA EXTRACT ON A CISPLATIN-INDUCED NEUROPATHIC MODEL

Keyong Ho Lee[1], Ki-Hyeong Rhee[2]

[1]CKD Research Institute, Chong Kun Dang Pharm., Yongin, 449-797, Republic of Korea
[2]College of Industrial Sciences, Kongju National University, Yesan 340-702, Republic of Korea

Corresponding Author: K. H. Rhee, College of Industrial Sciences, Kongju National University, Yesan 340-702, the republic of Korea.
E-mail: howard@kongju.ac.kr

Abstract

Background: Natural products including *Agrimonia eupatoria* are considered an incomparable source of molecular diversity that has led to the medicines, especially for pain treatment. To investigate the antinociception of *Agrimonia eupatoria*, we examined its activity in a rat model of cisplatin neuropathy.
Materials and Methods: Male Sprague-Dawley rats received intraperitoneal (i.p.) cisplatin twice a week at a dose of 2 mg/kg (cumulative dose, 20 mg/kg) for 4 weeks. Before each injection, 2 ml of sterile saline solution was given subcutaneously to prevent renal damage via hyperhydration. The mice were treated with gabapetin as a positive control drug with a 100mg/kg intraperitoneal injection. *A. eupatoria* extract of 200mg/kg was solved in saline and then treated by oral administration.
Results: The mice treated with *A. eupatoria* showed lower withdrawal duration in the pin-prick and plantar tests, and a higher withdrawal threshold in the paw-withdrawal threshold test as compared to control animals in a cisplatin-induced neuropathic model. In the case of cold-allodynia, A. eupatoria treatment increased paw-withdrawal duration in a chemical test. *A. eupatoria* showed a more outstanding effect than gabapentin in all used tests for preventing cisplatin-induced nerve injury for 4 weeks.
Conclusions: Our results suggest that *A. eupatoria e*xtract showed an antinociceptive effect in the pin-prick test, plantar test, and paw-withdrawal threshold test using a cisplatin-induced neuropathic rat model.

Key words: Agrimonia eupatoria, Cisplatin, Nociception, Hyperalgesia.

Introduction

Pain is a common but distressing symptom of many diseases. In general, analgesics relieve pain by acting on the central nervous system or peripheral pain mechanisms, without significantly altering consciousness (Stewart et al., 2003). Patients suffering from this kind of pain include those with so-called nociceptive and neuropathic pain, allodynia, and hyperalgesia (Koltzenburg & Scadding, 2001; Woolf & Doubell, 1994). However, pain management remains a major clinical challenge, because there is not an appropriate understanding of the mechanisms causing and maintaining pain or effective treatments (Dickenson, 1991).Neuropathic pain is usually difficult to treat because its etiology is heterogeneous and the underlying pathophysiology is complex. The clinical features of neuropathic pain include the paradoxical combination of sensory loss in the neuropathic area suffering from pain and hypersensitivity phenomena such as allodynia and hyperalgesia (Bennett GJ, 1994). Because no universally efficacious therapy exists for these nociceptive signs, a better understanding of the mechanisms responsible for these nociceptive signs is required to develop more specific therapies. Among toxic neuropathies, those that are a consequence of certain chemotherapies (paclitaxel, vincristine, and cisplatin) frequently involve the development of painful sensory disorders and sometimes require the cessation of anticancer therapy (Airiau, 1999; Windebank, 1999).

Cisplatin is used extensively in the treatment of cancers (testicular, ovarian, breast, bladder and lung), alone or in combination with another neurotoxic agent, paclitaxel, which is associated with serious adverse effects including nephrotoxicity and peripheral neurotoxicity. Renal toxicity is limited by chloride diuresis before, during, and after treatment. Sensory neuropathy, with complaints of paresthesiae and dysesthesiae in the distal extremities, is the major side effect (Krarup-Hansen et al., 1993).

In animal studies, behavioural assessments have shown sensory (thermal hypoalgesia) and motor (coordination and motor force decrease) disorders after repeated injections of cisplatin in rats and mice (Authier et al., 2000). Numerous neurophysiologic studies have shown that cisplatin decreases sensory nerve conduction velocities and reduces the amplitude of nerve action potentials, with minimal or no motor involvement (Boyle et al., 1999).Cisplatin may be expected to accumulate in dorsal root ganglia, leading to nucleolar damage and an alteration in peptide content, and would also exert its neurotoxic effects through Schawnn cells (Yamamoto et al., 1997). Therefore, it is necessary to search for new, effective, and safe analgesics among natural products derived from secondary metabolites.

The genus Agrimonia, belonging to the Rosaceae, include about a dozen species, which are perennial herbaceous flowering plants, mainly distributed in the temperate regions of the Northern Hemisphere. Some species have been used in traditional medicine, e.g. *Agrimonia eupatoria* has been traditionally used in Europe as an astringent, cholagogue, diuretic, and anti-diabetic agent (Shabana et al., 2003), *Agrimonia japonica* has also been used in Japan as an antidiarrheal and a hemostatic agent (Okuda et al., 1984), and *Agrimonia pilosa* has been used in Traditional Chinese Medicine (TCM). The roots and aerial parts of *A. pilosa* have also had different usage in TCM. Roots have been used for the treatment of cancer, and tannins have been reported to be

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principal constituents for this usage (Miyanoto et al., 1988). The aerial parts of *A. pilosa* were also listed in the Chinese Pharmacopoeia as an astringent hemostatic for treating various kinds of bleeding, including bloody dysentery, and also to counteract toxins and reduce swelling for treating boils and sores (Xu et al., 2005). Pharmacological studies on the extracts prepared from the aerial parts of *A. pilosa* demonstrated broad biological properties, such as anti-hemorrhagic (Wang et al., 1984), antiplatelet (Wang et al., 1985), antioxidant (Zhu et al., 2009), nitric oxide scavenging (Taira et al., 2009), acetylcholinesterase inhibition (Jung & Park, 2007), and a-glucosidase inhibition (Liu et al., 2014)and antinociception (Park et al., 2012). Chemical studies on the aerial parts of *A. pilosa* showed the presence of flavonoids (Jung & Park, 2007), 3,4-dihydroisocoumarins (Taira et al., 2009), and triterpenoids (An et al., 2005).

However, no studies have been made of the effect of *A. eupatoria* extracts on cisplatin-induced pain models, although the antinociceptive effect of *A. pilosa*was recently found in pain model. Therefore, the present study was designed to investigate whether *A. eupatoria* extracts exhibit antinociceptive effects on postoperative pain through various pain models including a cisplatin-induced model.

Materials and Methods

Animals

Seventy-two male Sprague-Dawley rats (Slc, Shizuoka, Japan), weighing 180 to 200 g at the beginning of the experiment, were used and housed in plastic cages on a 12h light/12h dark cycle with access to water and food *ad libitum*. Room temperature was maintained at 22°C and relative humidity was usually between 50 and 60%. Animals were allowed a 1-week acclimatization period before use in experiments. All experimental procedures used in the present study were approved by the Ethics Committee of Kongju National University which has adopted the guidelines established by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Test Sample

The aerial part of *A. eupatoria* (500 g) was extracted with a volume of 80% ethanol equal to ten times that the sample of *A. eupatoria* used for 48h at room temperature, and then the extract was filtered and evaporated under reduced pressure. A test sample of 200mg/kg was solved in saline and then treated by oral administration. The aerial part of *A. eupatoria* was purchased from Jung Do Herb Co. Ltd (Seoul, Korea). A voucher specimen which was authenticized by one of the authors (Prof. K. H. Rhee) was deposited at the herbarium of the college of Industrial Sciences, Kongju National University, Korea.

Cisplatin-Induced Neuropathic Pain Model

Cisplatin (10 mg/20 mL, Laboratoires Qualimed Levallois-Perret, France) was diluted in normal saline (0.9% NaCl) just before administration to a final concentration between 0.08 and 0.15 mg/mL, depending on animal weight, while ensuring that volumes of less than 5 mL would be injected into the peritoneal cavity. Injected volumes of saline (0.9% NaCl) were calculated according to the weight of the ratin the control group. Cisplatin was administered intraperitoneally twice a week at a dose of 2 mg/kg (cumulative dose, 20 mg/kg) for 4 weeks. Before each injection, 2 mL of sterile saline solution was given subcutaneously to prevent renal damage via hyperhydration. Gabapetin as a positive control drug was administrated with a 100mg/kg intraperitoneal injection.

Behavioral Assessment

Pin-prick test, paw-pressure test, plantar test and chemical test were conducted before each injection of cisplatin. The guidelines of the IASP Committee for Research and Ethical Issues were followed for all experiments (Zimmermann, 1983).

Pin-prick Test

Mechanical hyperalgesia was assessed by pin-prick test: Touching (not penetrating) the skin with the point of a safety pin. The surface of the injured hind paw was touched with the point of a bent gauge needle (at 90° to the syringe) at intensity sufficient to produce a reflex withdrawal response. The paw withdrawal duration was recorded in seconds and the normal quick reflex withdrawal response was given the value of 0.5s (Nakamura et al., 1996).

Paw-pressure Test

Response to noxious mechanical stimulation was determined by measuring the withdrawal threshold to paw-pressure using an Analgesimeter (Orchid Scientifics & Innovatives, Nashik, India). Continuously increasing pressure was applied to the dorsal surface of the affected hind paw using the blunt conical probe in Randal Selitto test instrument. On the day before the experiment, nociceptive thresholds of the right or left hind paw of each rat were measured 4 times at 1h intervals. Mechanical pressure was increased until withdrawal reflex occurred while the rats were lightly restrained. Withdrawal reflex threshold were expressed in grams (Randall & Selitto, 1957).

Plantar Test

The Hargreaves method was used to assess paw-withdrawal duration to thermal nociceptive stimulus. The preoperative pain duration of the animals was recorded before surgery. Radiant heat was applied from below to the plantar surface ofeach hind-

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paw and the withdrawal duration was measured with a stop-watch. Three measurements were performed oneach hindpaw using a plantar test device from Orchid Scientifics & Innovatives with at least 1min intervals to determine mean paw-withdrawal duration (PWD). A preliminary or control paw withdrawal threshold was measured for each rat before drug injection. The cut-off value was determined as 30s in order to avoid tissue damage (Hargreaves et al., 1988).

Chemical Test

The rats were housed in transparent plastic boxes with a floor made of 6mm wire mesh. After a 5-10min accommodation period, 0.5 mL of acetone was sprayed onto the plantar surface of the rat's hind leg from below the grid with a syringe with a blunt needle. Rats that developed cold allodynia withdrew the injured limb from the floor when acetone was applied, and the amount of time spent with the leg raised was used as a measure of pain. Both hind legs were tested in each animal, starting with the control leg, with an interval of 3min between them. The cut-off duration was determined to be 30s (Choi et al., 1994).

Statistical Analysis

Behavioural data were evaluated using analysis of variance (Fisher test) followed by Student's t test to detect either differences within treatment groups between baseline and post injection values or differences between each treatment group on each day tested. Investigators performing the behavioral and histologic studies were blinded with respect to the treatment received by each group.

Results

Effect of *A. Eupatoria* Extract on Mechanical Hyperalgesia

Cisplatin in rats induced neuropathic pain resulting in significant development of mechanical hyperalgesia in response to pin-prick stimulation and the application of pressure on the lateral surface of ipsilateral hindpaw. The paw-withdrawal duration to pin-prick significantly increased ($p<0.001$) and the paw-withdrawal threshold to the application of pressure significantly decreased ($p<0.001$) in the ipsilateral paw with the lapse of time. Treatment of cisplatin alone rapidly delayed the paw-withdrawal duration time as 6.24s at 1 week as compared to its duration time at 0 week of 0.89s, and then the paw-withdrawal duration time by cisplatin was increased gently to 8.22s by 4 week. The group treated with *A. eupatoria* extract of 200 mg/kg for 1 week showed an increase of duration time of4.5 times as compared to the7.0 times of the control group. Meanwhile, gabapentin also showed an increase of duration time of4.0 times as compared to 7.0 times of control group, it was a similar effect to *A. eupatoria* extract at 1 week. In the progress of time, the group of *A. eupatoria* extract maintains an inhibitory effect against increase of duration until the end of the experiment. In contrast gabapentin couldn't prevent the increase of duration time as it showed a rapid increase from 4.52s to 8.09s from 2 week to 4 week. According to these data, *A. eupatoria* extract is better able to inhibit the increase of paw-withdrawal duration time than gabapentin during the experimental period. Treatment of cisplatin alone rapidly decreased the pressure value of the paw-withdrawal threshold as much as 73g (from 241g to 168g) in the paw-withdrawal threshold at the point of 1 week and kept the value of 83g (from 241g to 158g) at the end of the experimental period. For improvement of the threshold value, *A. eupatoria* extract of 200 mg/kg treated and inhibited the decrease of the threshold value as much as 47g (from 234g to 187g) at 1 week. In this case, gabapentin inhibited a decrease of the threshold value of24g (from 236g to 212g) at the same point. With the progress of time, the group treated with *A. eupatoria* extract showed a value of decreased paw-withdrawal threshold of47g at 1 week and kept the same value at the end of the experimental period. In contrast, gabapentin didn't show a resistant activity of 95g which was increased from 236g at 0 week to 141g at 4 week. From these data, *A. eupatoria* extract have the potential activity to improve the paw-withdrawal duration time and threshold as compared to gabapentin (Table 1 and 2).

Table 1: Effect of *A. eupatoria* extracts on mechanical hyperalgesia using the pin-prick test on a cisplatin-induced pain models

Treatment	Paw-withdrawal Duration (sec)				
	0 week	1 week	2 week	3 week	4 week
Normal	0.86± 0.1	0.87± 0.2	0.90± 0.3	0.92± 0.1	0.90± 0.4
Control	0.89± 0.2	6.24± 0.5	7.98± 0.6	8.23± 0.4	8.22± 0.5
Gabapentin	0.91± 0.3	3.75± 0.3	4.52± 0.3	8.14± 0.3	8.09± 0.3
A. eupatoria extract	0.84± 0.3	3.81± 0.2	3.74± 0.3	4.97± 0.4	5.37± 0.3

Values are expressed Mean ± SEM, ($n=6$), $p<0.05$ compared to the control group. One way ANOVA followed by Tukey's Multiple Comparison Test.

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Table 2: Effect of *A. eupatoria* extracts on mechanical hyperalgesia using the paw-withdrawal threshold test on a cisplatin-induced pain models

Treatment	Paw-withdrawal Threshold (g)				
	0 week	1 week	2 week	3 week	4 week
Normal	245± 17	237± 10	243± 20	241± 19	238± 21
Control	241± 15	168± 14	162± 13	154± 11	158± 13
Gabapentin	236± 19	212± 20	172± 15	143± 9	141± 15
A. eupatoria extract	234± 18	187± 11	195± 13	193± 12	187± 10

Values are expressed Mean ± SEM, (n=6), $p<0.05$ compared to the control group. One way ANOVA followed by Tukey's Multiple Comparison Test.

Effect of *A. Eupatoria* Extract on Thermal Hyperalgesia

The group treated with only cisplatin showed a significant increase in withdrawal latencies of 0.83s, 10.4s, 11.6s, 12.3s and 12.8s from 0 week to 4 weeks ($p<0.05$). *A. eupatoria* extract of 200mg/kg showed a significant increase in withdrawal latencies of 3.2s at 1 week. However it didn't increase more than that until at the end of experiment. At the point of 1 week, the antihyperalgesia effect of *A. eupatoria* extract was 75%, compared to46% of gabapentin group. Those effects of both *A. eupatoria* extract and gabapentin were maintained until 4 week at 76% and 45%, respectively. However, *A. eupatoria* extract have shown superior antihyperalgesia activity when compared with gabapentin during the experimental period. Therefore, *A. eupatoria* extract has a greater potential to prevent thermal hyperalgesia by cisplatin than gabapentin (Table 3).

Table 3: Effect of *A. eupatoria* extracts on thermal hyperalgesia using the plantar test on a cisplatin-induced pain models

Treatment	Paw-withdrawal Duration (sec)				
	0 week	1 week	2 week	3 week	4 week
Normal	0.81± 0.2	0.84± 0.1	0.84± 0.2	0.80± 0.3	0.81± 0.2
Control	0.83± 0.3	10.4± 0.1	11.6± 0.3	12.3± 0.5	12.8± 0.3
Gabapentin	0.87± 0.3	4.5± 0.1	5.1± 0.1	6.1± 0.1	7.4± 0.1
A. eupatoria extract	0.84± 0.4	3.2± 0.3	3.5± 0.1	3.4± 0.1	3.7± 0.1

Values are expressed Mean ± SEM, (n=6), $p<0.05$ compared to the control group. One way ANOVA followed by Tukey's Multiple Comparison Test.

Effect of *A. Eupatoria* Extract on Cold-Allodynia Hyperalgesia

The behavioral response to acetone in the cisplatin-induced neuropathic group as a control was significantly reduced as compared to the normal group ($p<0.05$). The withdrawal latencies of the control group gradually and significantly showed decreased values of 15.3s, 10.4s, 7.3s, 5.7s and 4.9s over the course of 4 weeks. The withdrawal latency time of the *A. eupatoria* extract group rapidly dropped from 15.7s to 11.8s at 1 week, however its latency times were kept constantly at from 11.8s to 10.8s after 1 week. In contrast, the gabapentin group showed a more serious decrease than that of *A. eupatoria* extract group. At 1 week, the antihyperalgesia effect of *A. eupatoria* extract was 20% as compared to 8% of gabapentin. At the end of the experiment, the antihyperalgesia effect of *A. eupatoria* extract was increased to 53%, compared to 18% of gabapentin. These results present a

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potential antihyperalgesia effect of *A. eupatoria* extract to repair the neuropathic pain due to cisplatin (Table 4).

Table 4: Effect of *A. eupatoria* extracts on cold-allodynia hyperalgesia using the chemical test on a cisplatin-induced pain models

Treatment	Paw-withdrawal Duration (sec)				
	0 week	1 week	2 week	3 week	4 week
Normal	16.2± 0.3	16.0± 0.1	15.8± 0.3	15.7± 0.1	16.3± 0.4
Control	15.3± 0.4	10.4± 0.3	7.3± 0.1	5.7± 0.1	4.9± 0.2
Gabapentin	16.0± 0.2	11.5± 0.2	10.5± 0.2	9.1± 0.2	7.5± 0.3
A. eupatoria extract	15.7± 0.3	11.8± 0.2	10.8± 0.3	11.2± 0.3	10.8± 0.2

Values are expressed Mean ± SEM, (n=6), $p<0.05$ compared to the control group. One way ANOVA followed by Tukey's Multiple Comparison Test.

The cisplatin-induced neuropathic model is the representative animal model for inducing neuropathic pain. This model displays nociceptive signs such as a mechanical hyperalgesia and allodynia, a cold thermal hyperalgesia and allodynia, and a heat thermal hypoalgesia associated with significant electrophysiologic and histologic disorders (Authier et al., 2003).A major advantage of this model is persistent tactile allodynia. We used the pin-prick test and paw-withdrawal threshold test for mechanical hyperalgesia, and the plantar test for thermal hyperalgesia, and the chemical test for cold-allodynia in this study. According to various studies, such allodynia extended to at least 46 days by using cisplatin-induced mice. This persistency is in agreement with the dysaesthesia reported in the human chemotherapy patient (Authier et al., 2003; Krarup-Hansen et al., 2007; Ta et al., 2009).In the present study, we found that *A. eupatoria* extract produces antinociceptiive activity with various pain measure methods in cisplatin-induced rats. Our results demonstrate that *A. eupatoria* displayed lower withdrawal duration in the pin-prick test and plantar test, and a higher withdrawal threshold in the paw-withdrawal threshold test as compared to control animals in cisplatin-induced neuropathic model. In case of cold-allodynia, *A. eupatoria* increased paw-withdrawal duration in the chemical test. The positive control drug, gabapentin, showed a lower effect than *A. eupatoria* in all used test for preventing cisplatin-induced nerve injury. This study showed a very similar result with another research group that found the antinociceptive activity of *A. pilosa* extract on the acetic acid-induced writhing, tail-flick and hot-plate test (Park et al., 2012).*A. eupatoria* and *A. pilosa*are very similar herbs, even though they have been used differently and called by different names. *A. eupatoria* has been traditionally used in Europe as astringent, cholagogue, diuretic and antidiabetic agent (Shabana et al., 2003), and *A. pilosa*has been used for the treatment of cancer, and tannins were reported to be principal constituents for this usage (Miyanoto et al., 1988). Investigating each analytic component in methanol extract between *A. eupatoria* and *A. pilosa*, there are many common compounds, such as agrimony, agrimony lactone, tannin, flavonoids and glycosides. Therefore, this is the reason why *A. eupatoria* extract exhibited an antinociceptive effect that was similar to that of *A. pilosa*.Therefore, *A. eupatoria* extract has an antinociceptive effect in the neuropathic pain model, specifically, the chemotherapy-induced neuropathic pain model.

Conclusion

The findings of the present study demonstrate that *A. eupatoria* extract showed an antinociceptive property in a cisplatin-induced neuropathic pain model. *A. eupatoria* extract showed lower withdrawal duration in the pin-prick test and plantar test, and a higher withdrawal threshold in the paw-withdrawal threshold test as compared to control animals in a cisplatin-induced neuropathic model. In case of cold-allodynia, *A. eupatoria* increased paw-withdrawal duration in the chemical test. *A. eupatoria* extract showed superior activity to gabapentin in all used test for preventing cisplatin-induced nerve injury. Therefore, *A. eupatoria* extract is found to possess therapeutic potential for the treatment of neuropathic pain.

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