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Antinociceptive and Anti-Inflammatory Effects of *Lonicera japonica* Thunb

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Abstract: The present study was carried out to establish the antinociceptive and anti-inflammatory activities of the aqueous extract of *Lonicera japonica* flowers' buds in various experimental animal models. The antinociceptive activity was measured using the abdominal constriction, hot plate and formalin tests, while, the anti-inflammatory was measured using the carrageenan-induced paw edema. The dried flower's buds of *L. japonica* was added with distilled water (1:10 w v^{-1}) and boiled for 2 h at 80°C. The supernatant collected was freeze-dried overnight and prior to use was diluted to the desired doses. The extract (30, 100 and 300 mg kg^{-1}; administered intraperitoneally) exhibited significant ($p<0.05$) antinociceptive and anti-inflammatory activities in all assays used. In conclusion, the flower's buds of *L. japonica* possessed potential antinociceptive and anti-inflammatory activities that require further in-depth studies.

Key words: Lonicera japonica, flower's buds, aqueous extract, antinociceptive activity, anti-inflammatory activity

INTRODUCTION

Lonicera japonica Thunb, well known as *Japanese honeysuckle*, is distributed in China, Japan and Korea (Reid, 1987), but native to Japan (Williams and Timmins, 1988). Known among the Chinese as Jinyinhua, *L. japonica* is a plant that belongs to the family Caprifoliaceae. The plant is a slightly sweet, cooling herb that was commonly used in Chinese traditional medicine to treat various ailments (Ody, 1993). The flowers of *L. japonica* are widely used internally for acute rheumatoid arthritis, hepatitis, upper respiratory tract infections, dysentery, fever, throat inflammations, measles, chickenpox, infected wounds, gastroenteritis and breast cancer, to name a few. Externally, the flowers are used for skin inflammations, rashes and sores (Langley and Folkard, 2002).

Scientifically, the flowers of *L. japonica* have been reported to possess anti-inflammatory (Jin *et al*., 2003; Lee *et al*., 2001) and hepatoprotective (Ohta *et al*., 1993) activities. Through molecular studies, the anti-inflammatory activity of this plant was found to be mediated via inhibition of nuclear factor-κB (NF-κB) activation (Lee *et al*., 2001). In term of its phytochemical constituents, several reports have reported on the presence of essential oil (e.g., linaool, geraniol, aromadendrene and eugenol), saponins, terpenoids, flavones, phenolics, 3-caffeoyl-quinic acid and its metyl ester, 3, 5-dicaffeoyl-quinic acid and its methyl ester, irridoid glycosides, tannins and luteolin (Schlotzhauer *et al*., 1996; Duke, 1992; Li *et al*., 2000; Machida *et al*., 2002; Chevallier, 1996).

Although, there have been scientific studies on the flower of *L. japonica* anti-inflammatory activity, no pharmacological studies have been conducted on its antinociceptive activity despite both activities close connection in terms of the pathways and mediators involved. Thus, the present study was performed to determine the antinociceptive and anti-inflammatory activities of the aqueous extract of *L. japonica*.

MATERIALS AND METHODS

Plant material and preparation of Its aqueous extract: Dried *L. japonica* flower buds were bought from local Chinese medicine hall in Taman Sri Serdang, Serdang, Selangor, Malaysia. It was identified by Mr. Shamsul Khamis, a botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia.

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Approximately 1 kg of dried *L. japonica* flower buds were grinded into powder form added with distilled water (dH_2O) (1:10 w v^{-1}). The mixture was then boiled in flask with magnetic stirrer on the hotplate at 80°C for 2 h. The supernatant was filtered using Whatman No. 1 filter paper, collected and freeze-dried overnight. The dried extract obtained, labeled as LJAE, was dissolved in 0.9% normal saline before use.

Preparation of drugs: Ibuprofen (100 mg kg^{-1}) (Sigma, USA), acetylsalicylic acid (ASA; 10 mg kg^{-1}) (Sigma, USA) and morphine (5 mg kg^{-1}) (Sigma, Germany) were used as reference drugs and prepared by dissolving them in dH_2O.

Experimental animals: Male Balb-C mice (25-30 g; 5-7 weeks) and Sprague-Dawley rats (180-200 g; 8-10 weeks old), obtained from the Animal Source Unit, Faculty of Veterinary Medicine, University Putra Malaysia (UPM), Serdang, Selangor, Malaysia, were used in this study. All of the animals were kept under room temperature (27±2°C; 70-80% humidity; 12 h light/darkness cycle) in the Animal Holding Unit, Faculty of Medical and Health Sciences, UPM for at least 48 h before use. Food and water were supplied *ad libitum* up to the beginning of the experiments. At all times the mice and rats were cared for in accordance with current UPM principles and guidelines for the care of laboratory animals and the UPM ethical guidelines for investigations of experimental pain in conscious animals as adopted from Zimmermann (1983).

All mice were equally divided into 10 groups of 6 mice each (n = 6) and received (sc) dH_2O, ASA (100 mg kg^{-1}) or LJAE (30, 100 and 300 mg kg^{-1}) 30 min prior to subjection to the abdominal constriction or hot plate tests, respectively. On the other hand, all rats were equally divided into 11 groups of 6 rats each (n = 6). The first 6 groups were used in the formalin test and received (sc) dH_2O, 100 mg kg^{-1} ASA, 5 mg kg^{-1} morphine or LJAE (30, 100 and 300 mg kg^{-1}), respectively 30 min prior to subjection to the test. The second 5 groups were used in the nti-inflammatory study and received (sc) dH_2O, 100 mg kg^{-1} ibuprofen or LJAE (30, 100 and 300 mg kg^{-1}), respectively 30 min prior to subjection to the test. All of the test solutions were administered in the volume of 10 mL kg^{-1} body weight.

Antinociceptive assay

Abdominal constriction test: The abdominal constriction test which has been slightly modified (Zakaria *et al*., 2006) was used to evaluate the chemically-induced antinociceptive activity of LJAE.

Hot plate test: The 50°C hot-plate test which has been slightly modified (Zakaria *et al*., 2006) was used to evaluate the thermally-induced antinociceptive activity of LJAE.

Formalin test: The formalin test described by Hunskaar and Hole (1987) was used but with slight modifications. Pain was induced by injecting 2.5 μL of 25% formalin in the subplantar region of the left hind paw. Rats were given (sc) test solutions 30 min prior to formalin injection. The rats were individually placed in transparent Plexiglass cage observation chamber. The amount of time the animal spent licking the injected paw (Mendes *et al*., 2000), considered as an indicator of pain, was recorded for duration of 30 min following the formalin injection. The early phase of nociception, indicating a neurogenic type of pain response, was measured between 0-5 min, while the late phase of nociception, indicating an inflammatory type of pain response, was measured 15-30 min after formalin injection.

Anti-inflammatory assay: The carrageenan-induced paw edema test which has been slightly modified (Sulaiman *et al*., 2004) was used to determine the anti-inflammatory activity of LJAE.

Statistical analysis: The results are presented as Mean±Standard Error of Mean (SEM). The one-way ANOVA test with Dunnett post-hoc test was used to analyze and compare the data, with $p<0.05$ as the limit of significance.

RESULTS

Pharmacological studies on the LJAE: The LJAE antinociceptive activity assessed using the chemical-induced nociceptive assay was shown in Table 1. The 30, 100 and 300 mg kg^{-1} doses of LJAE significantly ($p<0.05$) reduced the acetic acid-induced writhing response in dose-dependent manner with the percentage of analgesia of 22, 53 and 78%, respectively. The 300 mg kg^{-1} dose of LJAE produced an equal effective activity when compared to the 100 mg kg^{-1} ASA, indicated by their insignificant percentage of analgesia (78 and 72%).

Table 1: Antinociceptive activity of LJAE assessed by the acetic acid-induced writhing test in mice

Treatment groups (n = 8)	Writhing response	Inhibition (%)
Saline	36±1.45	-
100 mg kg^{-1} ASA	10±0.71*	72
30 mg kg^{-1} LJAE	28±1.03*#	22
100 mg kg^{-1} LJAE	17±0.83*	53
300 mg kg^{-1} LJAE	8±0.72*#	78

The writhing response was expressed as mean±SEM; *: Data differs significantly ($p \leq 0.05$) when compared against the normal saline-treated group; #: Data differs significantly ($p \leq 0.05$) when compared against ASA-treated group

Table 2: Antinociceptive activity of LJAE assessed by the 50°C hot plate test in mice

	Latency of discomfort (sec)				
Treatment groups (n = 8)	0 min	30 min	60 min	120 min	180 min
Saline	6.22±1.02	6.92±0.60	8.38±1.09	7.78±1.10	7.83±0.92
5 mg kg^{-1} morphine	5.65±0.17	16.8±2.67*	18.88±2.48*	15.88±1.32*	13.18±1.24*
30 mg kg^{-1} LJAE	5.98±0.15	7.68±0.33§	9.40±0.36§	9.98±0.26§	10.28±0.26*
100 mg kg^{-1} LJAE	6.17±0.37	11.08±1.0*§	13.12±0.92*§	13.22±0.74*	11.93±1.14*
300 mg kg^{-1} LJAE	7.07±0.41	15.30±0.6*	16.68±0.71*	14.73±1.05*	11.75±0.52*

The latency for licking of the hind paws, shaking or jumping off from the surface was expressed as mean±SEM; *: Data differs significantly ($p \leq 0.05$) when compared against the normal saline-treated group; §: Data differs significantly ($p \leq 0.05$) when compared against morphine-treated group

Table 3: Antinociceptive activity of LJAE assessed by the formalin test in rats

	Licking time (sec)		Percentage of analgesia (%)	
Treatment groups (n = 5)	Early phase	Late phase	Early phase	Late phase
Saline	92.39±7.29$^{\#}$	57.64±2.25$^{\#}$	-	-
10 mg kg^{-1} morphine	16.22±0.88*	4.85±0.68*	82	92
100 mg kg^{-1} ASA	87.99±3.10*§	3.23±0.56*	5	94
30 mg kg^{-1} LJAE	52.89±3.99*$^{\S\#}$	29.74±4.58*$^{\S\#}$	43	48
100 mg kg^{-1} LJAE	40.95±5.77*$^{\S\#}$	20.93±2.22*$^{\S\#}$	56	64
300 mg kg^{-1} LJAE	26.14±4.01*$^{\S\#}$	5.72±0.96*	72	91

The licking time in both early and late phase was expressed as mean±SEM; *: Data differs significantly ($p \leq 0.05$) when compared against the normal saline-treated group; §: Data differs significantly ($p \leq 0.05$) when compared against morphine-treated group; $^{\#}$: Data differs significantly ($p \leq 0.05$) when compared against ASA-treated group

Table 4: Anti-inflammatory activity LJAE assessed by the carrageenan-induced paw edema test in rats

	Mean increase in paw edema±SEM (mL)/ Time interval (h)				
Treatment group (n = 6)	1 h	2 h	3 h	4 h	5 h
Saline	0.42±0.08	0.63±0.07	0.86±0.03	0.86±0.11	0.90±0.11
100 mg kg^{-1} ibuprofen	0.09±0.02*	0.12±0.01*	0.14±0.01*	0.09±0.02*	0.09±0.02*
30 mg kg^{-1} LJAE	0.11±0.02*	0.31±0.03*$^{\#}$	0.34±0.02*$^{\#}$	0.30±0.02*$^{\#}$	0.29±0.01*$^{\#}$
100 mg kg^{-1} LJAE	0.13±0.02*$^{\#}$	0.20±0.03*$^{\#}$	0.24±0.04*$^{\#}$	0.26±0.05*$^{\#}$	0.22±0.04*$^{\#}$
300 mg kg^{-1} LJAE	0.11±0.01*	0.15±0.02*	0.17±0.02*	0.16±0.02*$^{\#}$	0.14±0.02*
Inhibition (%)					
Saline	-	-	-	-	-
100 mg kg^{-1} ibuprofen	79	81	84	90	90
30 mg kg^{-1} LJAE	74	51	61	66	68
100 mg kg^{-1} LJAE	70	68	73	70	76
300 mg kg^{-1} LJAE	74	76	80	81	84

The volume of hind paw oedema was expressed as mean±SEM; *: Data differs significantly ($p \leq 0.05$) when compared against the normal saline-treated group; $^{\#}$: Data differs significantly ($p \leq 0.05$) when compared against ASA-treated group

The LJAE antinociceptive activity assessed using the thermal-induced nociceptive assay was shown in Table 2. The 30, 100 and 300 mg kg^{-1} doses LJAE significantly ($p<0.05$) increase the latency of discomfort 60 min after their administration and lasted until the end of the experiments. All doses of LJAE used were found to produce an activity that was lower than that of 5 mg kg^{-1} morphine.

The LJAE, at all doses used, also exhibited significant ($p<0.05$) antinociceptive effect in the early and late phases of the formalin test, which also occur in a dose-dependent manner (Table 3). The percentage of analgesia recorded for the 30, 100 and 300 mg kg^{-1} doses of LJAE were 43, 56 and 72% and 48, 64 and 91%, respectively. The 100 mg kg^{-1} ASA was only effective in the late phase while the 5 mg kg^{-1} morphine was effective in both phases of the formalin test.

The anti-inflammatory profile of the LJAE: The LJAE, at all doses tested, significantly ($p<0.05$) inhibited the edema development resulted from the carrageenan administration (Table 4). The activity also occurred in a dose-dependent manner and started after 1 h or their administration and lasted until the end of the experiment. The 100 mg kg^{-1} ibuprofen also shows the same pattern of activity.

DISCUSSION

The present study demonstrated the ability of LJAE to exert antinociceptive and anti-inflammatory activities in various animal models. The ability to inhibit chemically- and thermally-induced nociception indicates the extract's characteristic as strong analgesics (e.g., opioid agonists) (Hunskaar and Hole, 1987; Hosseinzadeh and Younesi,

2002). This is confirmed by the LJAE ability to inhibit both phases of the formalin test (Amanlou *et al*., 2005; Chan *et al*., 1995). In addition the ability to inhibit the thermal-induced nociception and the 2 phases of formalin test confirmed the extract's central antinociceptive activity (Pini *et al*., 1997; Amanlou *et al*., 2005).

Although, the exact mechanism of antinociceptive action of the LJAE is not yet determined, it is plausible to suggest the involvement of opioid receptor (Sulaiman *et al*., 2004) as part of the mechanism involved. Other than that, the involvement of Cyclo-Oxygenase (COX), at least at the peripheral level, is also suggested based on the LJAE potential to block the acetic-acid-induced nociception (Ballou *et al*., 2000; Vogel and Vogel, 1997). This suggestion is supported by findings that the abdominal constrictions induced by the acetic acid were due to the release of COX-synthesized prostacyclin (Ballou *et al*., 2000), PGE_2 and $PGE_{2\alpha}$ (Vogel and Vogel, 1997), which in turn lead to inflammatory pain within the peritoneal cavity. The above suggestion is further backed by the extract ability to exert anti-inflammatory activity when assessed by the carrageenan-induced paw edema test (Amanlou *et al*., 2005; Damas *et al*., 1986; Gamache *et al*., 1986).

Based on the previously determined chemical constituents of the flowers of *L. japonica* (Schlotzhauer *et al*., 1996; Duke, 1992; Li *et al*., 2000; Machida *et al*., 2002; Chevallier, 1996), it is plausible to suggest that the observed antinociceptive and anti-inflammatory of LJAE was attributed to the presence and synergistic action of flavonoids, triterpenes, saponins and tannins. These suggestions were supported by findings described by Kim *et al*. (2004), Beirith *et al*. (1999), Suh *et al*. (1996) and Starec *et al*. (1988).

CONCLUSION

In conclusion, the present study demonstrated the potential of *L. japonica* flowers' buds to exert antinociceptive and anti-inflammatory activities and thus, justify the folklore uses of the plant in treating pain-and inflammation-related ailments.

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