Asian Journal of Chemistry Vol. 20, No. 3 (2008), 1900-1906

Antiinflammatory and Antinociceptive Properties of Luteolin Diglucuronide and Apigenin Diglucuronide Obtained from *Perilla nankinensis*

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This study aimed to examine possible antiinflammatory and antinociceptive effects of luteolin diglucuronide (LT), apigenin diglucuronide (AP) and semi-pure luteolin diglucuronide (S-LT) obtained from *Perilla nankinensis* in rats and mice. Antiinflammatory effects of LT, AP and S-LT were investigated with the carrageenaan-induced rat paw edema tests. It was found that pretreatment with LT, AP and S-LT obtained from *Perilla nankinensis* reduced inflammagen -induced paw edema in rats. Again, pretreatment with LT significantly diminished the nociceptive response in mice. LT and AP present in the leaves of *Perilla nankinensis* may partly account for the antiinflammatory and antinociceptive properties of *Perilla nankinensis*.

Key Words: Analgesic, Antiinflammatory, Mouse, *Perilla nankinensis*, Rat.

INTRODUCTION

Perilla nankinensis (perilla, beefsteak plant), an edible and medicinal plant of Labiatae family, is native for Eastern Asia and is cultivated in many countries around the world. The leaves have a pleasant sweet taste and are used as a spice and combined with fish, rice, vegetables soups and salads in many Asian countries. It has been used for centuries in Oriental medicine as an antiasthmatic, antibacterial, antidote (in seafood poisoning), antimicrobial (influenza prevention), antipyretic, antispasmodic, antitussive, aromatic, carminative, antitumor, expectorant, stomachic and tonic[1-3]. Studies of the chemical composition of the *Perilla nankinensis*

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revealed the presence of caffeic-acid[1], terpene acids[2], catechin[3], ferulic acid[3], anthocyanins[4], apigenin[5], luteolin[6] and rosmarinic-acid[1]. The plant constituents confirm their uses in folk medicine and ongoing studies have revealed that this plant is useful in curing many cancers as well as various other diseases and disorders.

The inflammation response is usually initiated by cell injury or antigens, but its symptoms are related to released or synthesized chemical mediators such as histamine, serotonin, kinins, reactive oxygen species, prostaglandins and leukotrienes[7]. Vasoactive biogenic amines such as bradykinin, histamine and serotonin are some inflammation mediators and has been shown that they play a role in pathogenesis of inflammagen-induced inflammation in animal studies[8-10]. Non-steroidal antiinflammatory drugs (NSAIDs) exert their effects by inhibiting the metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways, the synthesis or release of inflammation mediators or their interactions[11,12].

It is believed that current analgesic and antiinflammatory drugs such as opiates and NSAIDs are not useful in all cases, prolonged use of these may leads to serious side-effects such as gastric intolerance, bone marrow depression, water and salt retention. For this reason, there is a need to find and develop a new antiinflammatory and analgesic drugs with low side-effect. Naturally originated new agents with little side-effects can replace chemical pharmaceuticals. Although its several therapeutic properties are well known, there is a lack of sufficient data about the antiinflammatory and anagesic activities of the *Perilla nankinensis*. The objective of this study was to determine the influence of luteolin diglucuronide (LT) and apigenin diglucuronide (AP) obtained from *Perilla nankinensis* on the inflammation and nociception, to compare antiinflammatory and antinociceptive potencies with those of conventional antiinflammatory and analgesic drug.

EXPERIMENTAL

Perilla nankinensis was collected during the blossom period of the plant, in the Ajara region of Georgia in 2002. It was identified by Dr. Dali Berashvili, Tbilisi State Medical University, Faculty of Pharmacy, Department of Pharmacognosy (Herbarium number: TGM, Voucher Number: 2118).

Preparation of extract: Dried and crushed leaves (250 g) were extracted three times using methanol 80 % (1:10). Yielded extracts were combined and thickened under vacuum till 0.2 L. The obtained precipitate was solved in 100 mL of water and cleaned of lypophilic substances by chloroform. Water phase was treated with ethyl acetate. Remained watery phase was evaporated and yielded the dark brown hygroscopic powder (33.5 g). 20 g of this powder was fractioned into the anion-Diaion HP 20 Mitsubishi Chemical Co, Tokyo, Japan. Water, 50 % methanol and pure

methanol were used for elution. Watery phase was lyophilized. 2 g of received powder was divided on liquid chromatograph Iobin-Ivon Chromato SPAC PREP 100, reverse phase RP 18. Again water and water-methanol solution was used for elution. From this fraction by using 30 % methanol, luteolin diglucuronide (LT) and by using 40 % methanol, apigenin diglucuronide (AP) were obtained. From the fraction received using 10 and 20 % methanol semi-pure luteolin diglucuronide (S-LT) was obtained.

Animals: Male albino Sprague-Dawley rats weighing 165-185 g and adult male albino mice weighing 32-37 g were used for the experiments. All animals were fed standard laboratory chow and tap water before the experiments. The animal laboratory was windowless with automatic temperature (22 ± 1 ºC) and lighting (14 h light/10 h dark) controls. Food and water were available *ad libitum*. There were six animals in all groups, which were housed in separate cages.

Drug and chemicals: Carrageenan (Sigma, USA), indomethacin (Deva, Turkey), were used for the experiments. All drugs were dissolved in a saline solution. All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

Carrageenan-induced paw edema in rats: All the drugs were given at 0.5 mL. LT, AP and S-LT were administered by gavage in doses of 1 and 2.5 mg/kg, with indomethacin at a dose of 20 mg/kg and the rats in the control group received the saline at the same volume. After 1 h, final drug administration, carrageenan 0.1 mL (1 %, w/v) solution was subcutaneously injected into the plantar surface of the right hind paw. The paw volume was measured with a plethysmometer before injection and four times at 1 h intervals. The antiinflammatory activity in animals that received LT, AP, S-LT were compared with that in the control groups. Per cent inhibition of paw edema was calculated using the relation:

$$\text{Inhibition of edema (\%)} = 100 \times [1 - (b/a)]$$

where a = mean paw volume of control rats; b = mean paw volume of treated rats.

Acetic acid-induced writhing test in mices: Saline, LT at doses of 2 and 5 mg/kg and indomethacin at a dose of 20 mg/kg were administered by gavage (at 0.5 mL). After 1 h treatment, the mice were injected intraperitoneally with 0.6 % (v/v) acetic acid solution (0.1 mL/10 g body weight) to induce the characteristic writhings. The number of writhings occuring between 10 and 20 min after acetic acid injection was counted. The values from treated group were compared with those of control (saline) and indomethacin groups. Per cent inhibition of writhing was calculated using the relation:

$$\text{Inhibition of writhing (\%)} = 100 \times [1-(b/a)]$$

where a = mean writhing number of control rats; b = mean writhing number of treated rats.

Statistical analysis: Values were presented as mean ± SEM and were analyzed by one-way analysis of variance (ANOVA), followed by the post hoc Tukey test. $p < 0.05$ accepted as statistically significant.

RESULTS AND DISCUSSION

Subplantar injection of carrageenan in rats showed time dependent increase in paw volume (Table-1). This increase was observed at 1 h and was maximal at 2 and 3 h after injection of carrageenan in the vehicle treated groups. However, carrageenan-induced inflammation was significantly reduced in first three phases of the experiment by treatment with LT 2.5 mg/kg. Carrageenan-induced paw volume changes are presented in Table-1. LT at dose of 2.5 mg/kg decreased the inflammatory response by 63.4 % ($p < 0.01$), 57.2 % ($p < 0.01$), 58.3 % ($p < 0.05$) and 37.5 % at 1, 2, 3 and 4 h after induction of edema, respectively. Again, 2.5 mg/kg dose of S-LT significantly ($p < 0.05$) decreased the inflammatory response at 1 and 2 h after induction of edema. On the other hand, significant inhibitor effect of indomethacin on the carrageenan-induced paw edema continued after 4 h.

TABLE-1
EFFECTS OF AP, LT, S-LT AND INDOMETHACIN ON CARRAGEENAN-INDUCED RAT PAW EDEMA

Treatment	Dose (mg/kg)	Edema rate percentage (mean ± SEM)			
		1 h	2 h	3 h	4 h
Control (saline)	–	29.80 ± 4.4	43.9 ± 6.3	43.4 ± 7.9	25.1 ± 3.5
Indomethacin	20.0	15.10 ± 4.9*	7.7 ± 2.9‡	15.4 ± 5.1†	8.8 ± 3.8†
AP	1.0	34.70 ± 7.1	43.9 ± 9.1	51.9 ± 11.7	43.5 ± 9.8
AP	2.5	12.90 ± 4.8*	26.4 ± 9.0	25.8 ± 7.6	35.9 ± 11.6
LT	1.0	22.10 ± 7.3	24.0 ± 5.0*	25.3 ± 6.8	29.9 ± 9.8
LT	2.5	10.90 ± 2.4†	18.5 ± 4.7†	18.1 ± 5.2*	15.7 ± 4.0
S-LT	1.0	23.00 ± 6.8	27.9 ± 7.7	37.0 ± 12.1	39.1 ± 9.2
S-LT	2.5	14.94 ± 2.8*	22.3 ± 6.9*	22.0 ± 8.5	21.2 ± 5.8

AP = Apigenin diglucuronide, LT = Luteolin diglucuronide, S-LT = Semi-pure luteolin diglucuronide.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$, *vs.* control, by ANOVA.

The inflammation response is usually initiated by cell injury or antigens, but its symptoms are related to released or synthesized chemical mediators such as histamine, serotonin, kinins, reactive oxygen species, prostaglandins and leukotrienes, which is frequently associated with pain. Inhibitor effects on cyclooxygenase and lipoxygenase products synthesis, release of inflammation mediators and free radicals are considered in evalu-

ation of the non-steroidal antiinflammatory drug activity. There are several experimental protocols for evaluating of antiinflammatory and analgesic potency of drugs. In the present study, the evaluation of anti-inflammatory and analgesic effects was undertaken using carrageenaan-induced paw edema test and acetic acid-induced writhing test.

Carrageenan-induced edema is an experimental acute inflammation model and is useful for the screening of the new antiinflammatory agents. Subcutaneous injection of carrageenan into the rat paw produces plasma extravasation and inflammation characterized by increased tissue water and plasma protein exudation with neutrophil extravasation and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways[13,14]. There are biphasic effects in carrageenan-induced edema. The first phase begins immediately after injection and diminishes in 1 h. The second phase begins at 1 h and remains through 3 h[9,14,15]. It is suggested that the early hyperemia of carrageenan-induced edema results from the release of histamine and serotonin[16]. On the other hand, the delayed phase of carrageenan-induced edema results mainly from the potentiating effect of prostaglandins on mediator release, especially of bradykinin and neutrophil-derived free radicals[17]. Some of antiinflammatory drugs strongly inhibit the second phase of carrageenan-induced edema. Hovewer, some antiinflammatory drugs are effective against both phases[9,18]. In the present study, LT (2.5 mg/kg) exerted significant anti-edematogenic effects on paw edema induced by carrageenan at 1-3 h. On the other hand, 2.5 mg/kg dose of S-LT inhibited significantly the formation of the carrageenan-induced rat paw edema at 1-2 h. Significant anti-edema effects of AP (2.5 mg/kg) and LT (1 mg/kg) were observed only at the first and second h, respectively. Indomethacin inhibited significantly the carrageenan-induced paw edema at 1-4 h. Depends on these results, it could be argued that anti-edematogenic effects of the *Perilla nankinensis* may be related to inhibition of inflammation mediators formation by LT and AP. Again, LT may be effective in both early and late phases, but AP may be effective only early phase. Luteolin is also reported to inhibit NO production, active oxygen species, tumour necrosis factor-α (TNF-α) production and metallo-peptidases[1]. Those activities of luteolin may account for antiinflammatory action. It has been revealed that crude extract of perilla leaf inhibited the arachidonic acid-induced ear edema[19]. Apigenin and especially luteolin may be responsible two active constituents of perilla against arachidonic acid-induced ear edema.

Table-2 shows the writhing response, which was presented as cumulative abdominal stretching response. The treatment of mice with LT (2 and 5 mg/kg, p.o.) produced a significant ($p < 0.001$) inhibition in abdominal writhes produced by acetic acid. The inhibition by LT (2 and 5 mg/kg) was

nearly similar to that produced by indomethacin (20 mg/kg, p.o.), used as a standard drug. The effects of LT doses were found to be more potent gravimetrically than that of indomethacin.

TABLE-2
EFFECTS OF LUTEOLIN DIGLUCURONIDE (LT) AND INDOMETHACIN ON WRITHING INDUCED BY ACETIC ACID IN MICE

Treatment	Dose (mg/kg)	Writhing number[a]	Inhibition (%)	p value
Control (saline)	–	21.2 ± 1.0	–	–
LT	2	4.5 ± 0.7	78.8	0.001
LT	5	4.7 ± 2.8	77.8	0.001
Indomethacin	20	5.3 ± 1.6	75.0	0.001

[a] mean ± SEM

In acetic acid-induced abdominal writhing which is visceral pain model, procesor release of arachidonic acid metabolites *via* cyclooxygenase and prostaglandin biosynthesis play a role in nociceptive mechanism[20]. High levels of prostaglandins $PGE_{2\alpha}$ and $PGF_{2\alpha}$ was determined during the first 0.5 h after acetic acid injection[21]. On the other hand, intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators[21]. Results of this study showed that 2 and 5 mg/kg doses of luteolin produced significant antinociceptive effect and this effect was very close to 20 mg/kg dose of indomethacin. Thus, the results obtained for the writhing test using acetic acid are similar to those obtained for the edematogenic test using carrageenan, since LT (2.5 mg/kg) was effective in inhibiting the acetic acid-induced writhing in mice. Based on the results of this study, we suggested that the antinociceptive effect of LT may be attributed to inhibition of prostaglandin release and other mediators involved in this test.

In conclusion, the results of this study demonstrated that AP and S-LT are slight and LT is a potent inhibitor of acute inflammation. Again LT has an antinociceptive activity. On the basis of the results obtained, these effects of AP, LT and S-LT on inflammagen-induced edema and chemical-induced nociception may be related to inhibition of the formation of several inflammation mediators. Luteolin diglucuronide may provide new alternatives for the clinical management of the inflammatory and painful disease. However, the mechanism(s) of the antiinflammatory and nociceptive effects of these substances in this are not yet clear. Further studies are require to elucidate the mechanisms behind traditional effects of *Perilla nankinensis*.

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(*Received*: 10 March 2007; *Accepted*: 2 November 2007) AJC-6059