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Anti-inflammatory Effects of *Labisia pumila* (Blume) F. Vill-Naves. Aqueous Extract

(Kesan Ekstrak Larutan *Labisia pumila* (Blume) F. Vill-Naves. Sebagai Anti-radang)

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ABSTRACT

This study was carried out to evaluate the anti-inflammatory effects of three concentrations of Labisia pumila *(Blume) F. Vill-Naves aqueous leaf extract in rats. The effects of these extracts as anti-inflammatory agents were determined using two experiments namely formalin-induced paw licking and carrageenan-induced paw oedema test. The exposure of inflammation to various treatments resulted in significant differences between treatments in formalin-induced paw licking in rats experiment whereas in phase 2, 50 mg kg^{-1} of* L. pumila *extract showed the most significant inhibition of 82.12%, followed by 10 mg kg^{-1} with 76.00% and 25 mg kg^{-1} with 57.80%. Similarly, different treatments showed significant effects at* $p<0.05$ *in the carrageenan inducing paw oedema experiment. All treatments were able to suppress the oedema formation induced by carrageenan as compared with the control. It is evident that the anti-inflammtory effect of every concentration of* L. pumila *extract started as early as the first hour of carrageenan injection and showed the maximum inhibition during the fifth hour. Again, 50 mg kg^{-1} of* L. pumila *extract was found to be the best treatment that could reduce inflammation with highest inhibition of 64.59% followed by 25 mg kg^{-1} with 56.99% and 10 mg kg^{-1} with 5.55%. The result of this study has shown that these extracts of* L. pumila *can be effective for anti-inflammation purposes which supports and justifies traditional uses of this plant.*

Keywords: Anti-inflammation; carrageenan induce paw oedema test; formalin-induced paw licking test; Labisia pumila; *medicinal plants*

ABSTRAK

Kajian ini telah dijalankan untuk menilai kesan tiga kepekatan ekstrak larutan Labisia pumila *sebagai anti-radang ke atas tikus. Kesan ekstrak sebagai anti-radang dikenal pasti melalui uji kaji formalin yang menyebabkan tikus menjilat tapak kaki dan karaginan yang menyebabkan kebengkakan pada tapak kaki. Kesan rawatan terhadap keradangan menunjukkan perbezaan bererti dalam pengurangan radang di dalam uji kaji formalin dan di dalam fasa 2, 50 mg kg^{-1} ekstrak* L. pumila *menunjukkan kesan pengurangan paling bererti sebanyak 82.12%, diikuti 10 mg kg^{-1} dengan 76.00% dan 25 mg kg^{-1} dengan 57.80%. Rawatan yang berbeza juga memberikan kesan yang bererti pada* $p<0.05$ *di dalam uji kaji karaginan yang menyebabkan kebengkakan tapak kaki. Semua rawatan yang diberikan dapat mengurangkan kebengkakan yang disebabkan oleh karaginan jika dibandingkan dengan kawalan. Adalah terbukti bahawa kesan anti-radang dalam setiap kepekatan ekstrak* L. pumila *bermula seawal jam pertama dan memberi kesan maksimum dalam pengurangan radang semasa atau pada jam kelima selepas suntikan karaginan. Sekali lagi kepekatan 50 mg kg^{-1} merupakan rawatan terbaik untuk mengurangkan radang dengan peratusan tertinggi sebanyak 64.59% diikuti dengan 25 mg kg^{-1} sebanyak 56.99% dan 10 mg kg^{-1} sebanyak 5.55%. Keputusan kajian menunjukkan bahawa ekstrak* L. pumila *adalah berkesan untuk digunakan sebagai rawatan anti-radang dan ini menyokong penggunaan tradisi tumbuhan ini.*

Kata kunci: Anti-radang; Labisia pumila; *tumbuhan ubatan; ujian jilat tapak kaki rangsangan formalin; ujian kebengkakan tapak kaki rangsangan karaginan*

Introduction

Medicinal plants are known to be used extensively as remedies for human health since ancient times. The reason for this is the presence of active chemical compounds which have properties as antioxidants, anti-bacterial and anti-inflammatory activities (Asmawi et al. 1993; Martınez Ruiz et al. 2012; Nair et al. 2012). In Malaysia, *Labisia pumila* (Blume) F. Vill-Naves is one of the most popular traditional medicinal plants known to have good effect on women's health. It is a small sub-herbaceous plant with creeping stems from the genus of *Labisia* that belongs to the family Myrsinaceae. *L. pumila* is commonly found in shady areas at 80 to 100 m elevations above mean sea level (Burkill 1935) and its natural distribution covers most areas of the South East Asian region including Malaysia, Thailand and Indochina. It is locally known as 'Kacip Fatimah' and sometimes

also referred as *selusoh Fatimah, rumput Siti Fatimah, akar Fatimah, tadah matahari, bunga belangkas hutan* and *pokok pinggang*. The species has three varieties in Malaysia, namely, *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *lanceolata* (Stone 1989). *L. pumila* has been commonly linked and acknowledged to be effective in curing many ailments such as post-partum treatment, anti-flatulence, rheumatism and preventing osteoporosis caused by post menopause (Jamia et al. 2003; Nadia et al. 2012). The values of this species as protection from disease are largely influenced by its phytoestrogen, anti-inflammatory and antioxidative properties (Nadia et al. 2012).

Inflammation is a protection mechanism as a reaction to injuries leading to the build-up of oedema (Sosa et al. 2002). Several medications such as prostaglandins and leukotrienes are known to influence pathophysiological pathway that cause inflammation (Londonkar et al. 2010). Thus, the anti-inflammatory aspect of this reaction are indeed important. Recent studies on *L. pumila* also reported the existence of anti-oxidant activities (Ibrahim & Jaafar 2011; Norhaiza et al. 2009), anti-bacterial activities (Karimi et al. 2009) and flavonoids and phenolic acids (Chua et al. 2011; Ibrahim & Jaafar 2011). In addition, its extract can also be used to treat inflammation caused by Ultra Violet Type B irradiation (UVB) (Choi et al. 2010). The evidence of established phytochemicals and bioactive compounds can be use as indicator of the usefulness of this important medicinal plant for future medication (Karimi & Jaafar 2011). With the increasing demands of this plant as a medicinal product; expansion and improvement on the knowledge of more efficient anti-inflammatory agent are directly needed. Therefore, information on scientific values and understanding of the use of *L. pumila* to overcome many ailments especially its anti-inflammatory effect is yet to be verified. Thus, this research has evaluated the anti-inflammatory effects of *L. pumila* using two different pharmacological experiments in an attempt to validate the presence of this activity.

Materials and Methods

Animals

The male Sprague Dawley Rats (weighing 120-290 g) were provided by the animal house of the Faculty of Medicine and Health Sciences of Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The animals were housed in cages where they were nourished with free access to standard laboratory food and tap water throughout the experiment. They were maintained at normal room temperature of 25 ± 2°C with 12 h light-dark cycle. All the experiments were conducted in accordance with the ethical guidelines on animal experimentation, approved by the Animal Care Unit Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (ACUC_UPM, FPSK, PADS, BR-UUH, 00330).

Plant Material

Only one variety of *L. pumila* var. *pumila* was selected and used in this study. The mature leaves were collected from thirty potted plants which were acclimatized at the nursery of the Faculty of Forestry, Universiti Putra Malaysia. The potted plants were initially propagated vegetatively via cuttings in the nursery of the Forest Research Institute of Malaysia (FRIM). The origin of these plants was from Sungai Nipah Forest Reserve, Kemaman, Terengganu, Malaysia.

Aqueous Extract Preparation

Prior to extraction, the selected fresh leaves were collected and washed thoroughly under running tap water. The leaves were then dried inside the oven at 40°C for 48 h; cut into smaller pieces before being pulverized coarsely into powder form using a grinder. The powdered mixture was kept in a sealed plastic bag until it was used for the testing experiment. The crude extract of the plant was obtained using the aqueous extraction method, where the powdered leaves were soaked in distilled water with 1:10 ratio. The solution was incubated in the water bath at 55°C for 48 h under atmospheric pressure, then filtered using Whatman filter paper No 1. and poured into the 50 mL centrifuge tubes. The solvent of extract was placed in the freezer at -80°C and was later put in freeze dryer to produce dried powder stock (solutes). From the stock, three different dosages of 50, 25 and 10 mg kg^{-1} were made to prepare the extract concentrations. Doses selected were based on reported pharmocological dosages which produced favourable effects without having adverse effects on rats. Effects of the anti-inflammation were tested using a starting concentration of 50 mg kg^{-1}. Two other concentrations tested were 10 and 25 mg kg^{-1}. These concentrations were then used for the anti-inflammation studies.

Anti-Inflammatory Study

Formalin-Induced Inflammation Test

Five groups of rats were treated with normal saline, acetyl salicylic acid (A.S.A) and three doses (10, 25 and 50 mg kg^{-1}) of *L. pumila* extracts, 30 min before 0.05 mL of 2.5% formalin were injected to the dorsal surface of the right hind paw. The rats were then placed back in their respective cages to observe their behavior against treatments. The period of time they spent licking the injected paw was considered as indicative of pain. They were recorded in two phases; the first phase was 0-5 min and the second phase 15-30 min after formalin injection. The time spent on licking the injected paw in both phases was recorded. Percentage of inhibition of inflammation was calculated using the following formula:

$$\% \text{ inhibition} = 100\ (1\text{-}T_1/T_C),$$

where, T_1 is the licking time in treatment and T_C is the licking time in control.

CARRAGEENAN INDUCED PAW OEDEMA TEST

The rats were divided into five groups where each group consisted of six male rats. The right hind paws of the rats were injected with 0.1% carrageenan. They were then treated with normal saline solution, A.S.A and 10, 25 or 50 mg kg^{-1} *L. pumila* extract. Increased volume of the right hind paws was taken as a sign of paw oedema. Paw volume was determined by volume displacement technique using a plethysmometer according to the following periods: just before the administration of carrageenan (initial volume), 1, 2, 3, 4 and 5 h after carrageenan injection (final volume). The paw oedema was determined using the following formula:

Oedema = Final volume – initial volume.

The percentage of inhibition of inflammation is calculated by using the formula:

$$\% \text{ inhibition} = 100\ (1 - V_1/V_C),$$

where, V_1 is the oedema volume in treatment and V_C is the oedema volume in control.

Statistical Analysis

Statistical analyses were performed using Statistical Analysis System (SAS) package version 2009. The data were expressed as mean±standard error of mean (S.E) and analyzed for their variances. Possible treatment variations were evaluated using the Duncan Multiple Range test. In all data analyses those differing at a probability of <0.05 were considered to be significantly difference.

Results and Discussion

Tissue damage and injury are always associated with pain and inflammation. In this formalin test, the rats used were treated with several treatments to reduce inflammation. Formalin test is a biphasic response where first phase is the direct effect of formalin which involves neurogenic pain. The pain is usually initiated when harmful mechanical, thermal or chemical stimuli agitate the peripheral terminals of particular main afferent neuron named nociceptors (Tominaga et al. 2004). The second phase is involved in the inflammatory reactions. In our study, we noticed that exposure of inflammation to various treatments resulted in a significant inhibition of inflammation. The results of mean paw licking time when treated with *L. pumila* extracts as compared with the controls between each phase are shown in Table 1. There were significant effects in every concentration of *L. pumila* extracts but phase 2, 50 mg kg^{-1} showed the most significant inhibition followed by 10 and 25 mg kg^{-1}. Phase 2 was found to significantly suppress the inflammation with high inhibition when treated with different concentrations. Fifty mg kg^{-1} gave the highest inhibition with 82.12% followed by 10 mg kg^{-1} with 76.00% and 25 mg kg^{-1} with 57.80% (Figure 1). The results showed that the given treatments of A.S.A and the three doses of *L. pumila* induced different licking frequencies. Perhaps, the duration of at least 15 min in phase 2 was sufficient to allow the rats to respond differently to different treatments. According to Okechukwa and Ikujuni (2012), activation of local inflammatory mediators is inhibited by the methanol leaf extract (MELP) and dichloromethane leaf extract (DELP) of *L. pumila* which concur with the result of our study. On the other hand, Hosseinzadeh and Younesi (2002) also found positive effects of anti-nociceptive and anti-inflammatory effects of *Crocus sativus* L. (saffron) stigma and petal extracts in mice. They concluded that both aqueous and ethanolic extracts of saffron stigma and petal have an antinociceptive effect, as well as acute and/or chronic anti-inflammatory activity.

According to Campos et al. (1995) and Gepdiremen et al. (2005), models induced by pro-inflammatory agents such as carrageenan, dextrane, formaldehyde, serotonin, histamine and bradykinin in rat paws are used to examine their influence on the acute inflammation activity. Carrageenan induces acute inflammatory activity which is useful for the detection of anti-inflammatory agents. From this result, the treatment might engage prostaglandin biosynthesis pathway and influence the other mediators of swelling as well. Since *L. pumila* extract could significantly reduce rat's paw oedema, it indicates that this extract has the ability to efficiently reduce acute inflammation. The early phase of the carrageenan response is due to the release of serotonin and histamine in the surrounding damaged tissues. In the following phase, bradykinin, protease,

TABLE 1. Mean paw licking time (s)±S.E in formalin-induced paw licking test

Treatment	Mean paw licking time(s)±S.E.M	
	PHASE 1	PHASE 2
Normal saline	23.23±2.31	33.00±1.61
A.S.A	22.46±1.35	20.05±1.38
50 mg kg^{-1}	22.67±2.49	5.87±1.23*
25 mg kg^{-1}	15.00±2.34	13.95±3.3*
10 mg kg^{-1}	20.35±2.01	7.92±1.06*

* = Significantly different at $p \leq 0.05$ compared with the control group

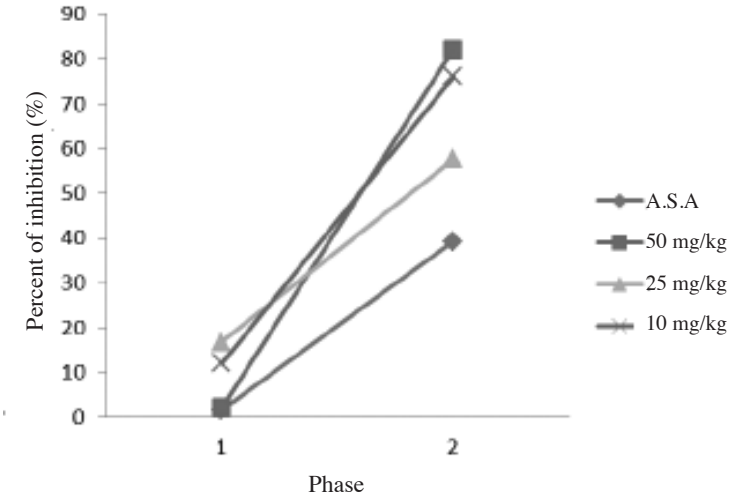

FIGURE 1. Percentage of paw licking time inhibition at different doses

prostaglandin and lysozomes are released. For anti-inflammatory agents, the time to react during the second phase is 3 to 5 h after being treated with carrageenan. The results from Table 2 shows that the administration of *L. pumila* at different concentrations decreases the oedema induced by carrageenan. The anti-inflammtory effect of *L. pumila* extract was evident in every concentration of the extracts as early as the first hour of carrageenan injection and maximum inhibition was during the fifth hour. It maintained the suppression of the inhibition throughout the duration of the study. This indicates that the plant extract may hinder any or all processes of inflammation and act as an anti-inflammatory substance. Generally, the percentage of carrageenan inhibition paw oedema is high in almost every treatment used. This indicates that the treatment especially *L. pumila* aqueous extract in the dose ranging from 10-50 mg kg^{-1} could significantly reduce swelling that was induced by carrageenan. The results of oedema inhibition from carrageenan test when treated produced the highest inhibition of 64.59% for 50 mg kg^{-1} followed by 25 mg kg^{-1} with 56.99%, A.S.A. with 23.72% and 10 mg kg^{-1} with 5.55%. The findings of this test showed a dose dependent pattern in which, the best treatment that could reduce inflammation was 50 mg kg^{-1} followed by 25 mg kg^{-1}, acetyl salicylic acid (A.S.A) and 10 mg kg^{-1} (Figure 2). Similar conclusion was drawn by Ibrahim and Jaafar (2011) who reported that the effectiveness of these extracts increase with concentrations. This might be due to the production of higher quantity of inflammatory components which in turn stimulate accumulation of protein-rich fluid at the oedema site causing better reduction in inflammation. In comparison with the standard drug of the A.S.A, the 10 mg kg^{-1} extract was less effective but was still able to reduce the oedema. The potential of medicinal plants to have anti-inflammatory effects have been reported in *Caralluma attenuata* (Ramesh et al. 1998), *Crinum asiaticum* (Samud et al. 1999), *Elephantopus tomentosus* (Yam et al. 2009), *Adenanthera pavonina* (Ara et al. 2010) and *Bryophyllum pinnatum* (Afzal et al. 2012). According to the study done by Hosseinzadeh and Younessi (2002), the stigma extract of *Crocus sativus* had the activity against inflammation. The extract taken from the leaves has the highest level of anti-inflammatory contents (Ibrahim & Jaafar 2011). The plant extract of this species that contained flavonoids, tannins and antocyanins did give the effects on the plant's anti-inflammation. The anti-inflammatory activity of *L. pumila* plant may be related to the presence of these chemical constituents. Moreover, a few studies have also showed that a variety

TABLE 2. Mean increase in paw volume (mL) ±S.E due to the Carrageenan-induced oedema in rats

Treatment	Mean increase in paw volume(mL)±S.E.M					
	initial	1 h	2 h	3 h	4 h	5 h
Normal saline	0	0.85±0.13	0.51±0.17	0.48±0.12	0.41±0.16	0.39±0.19
A.S.A	0	0.33±0.12*	0.37±0.13*	0.38±0.15*	0.31±0.10*	0.30±0.11*
50 mg kg^{-1}	0	0.10±0.05*	0.13±0.18*	0.15±0.09*	0.05±0.05*	0.03±0.07*
25 mg kg^{-1}	0	0.30±0.12*	0.15±0.08*	0.15±0.04*	0.11±0.06*	0.09±0.08*
10 mg kg^{-1}	0	0.49±0.42*	0.46±0.24*	0.47±0.33*	0.40±0.34*	0.39±0.34*

* = Significantly different as compared with the control group

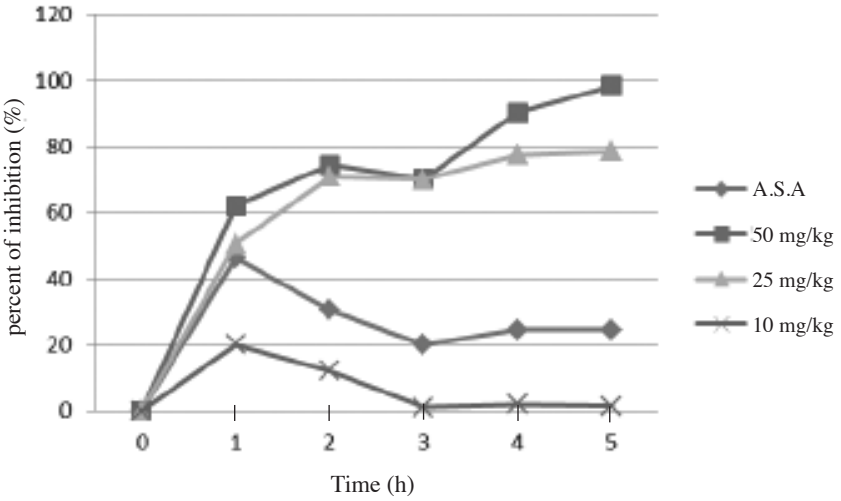

FIGURE 2. Percentage of paw oedema inhibition in different doses at particular period of time

of flavonoids could produce significant anti-inflammatory activities for example rutin, hesperidin and biflavonoids (Calixto et al. 2000; Ramesh et al. 1998). Therefore, it can also be suggested that *L. pumila* extract possesses high concentration of flavonoids and thus gave similar anti-inflammatory effect just like *Crocus sativus* plant extract. Besides the anti-inflammatory property, phenolic compounds also possess certain degree of anti-oxidant properties (Ibrahim & Jaafar 2011).

CONCLUSION

It could be concluded that aqueous extract of *L. pumila* has potent anti-inflammatory activity in rats in a dose dependent manner. The mechanism of anti-inflammation by *L. pumila* might be related with the compounds of bioactive and phytochemicals present in the plant. Therefore, this medicinal plant has the prospect to be used as herbal remedy for anti-inflammation. However, the study should be replicated and carefully tested against human cells to know the level of toxicity and its effects.

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