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The Study of Antinociceptive Effects of MEOH and DCM Crude Extracts from the Leaves of *Labisia pumila*

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Abstract: *Labisia pumila, LP* (Myrsinaceae), is a popular herb among the women in Malaysia known locally as ''Kacip Fatimah". Traditionally, the water decoction of the root and the whole plant is consumed by the Malay women for induction and facilitation of labour. Scientific studies have established that the plant possesses medicinal properties and biological activities. This study was aimed to investigate the antinociceptive effects of two different extracts methanol (MeOH) and dichloromethane (DCM) of different concentrations (50, 100 and 300 mg/kg) obtained from the leaves of *LP*, against two noxious stimuli, thermal (Hot plate) and chemical (Formalin). *LP* extracts of MeOH and DCM at different concentrations showed increased in the maximum possible effect against hot plate test. Similarly, in the formalin test, MeOH and DCM at varying concentration inhibit both the early and late phases. Phytochemical tests showed presence of tannins, saponins, flavanoids, steroids and terpenoids which have contributed to the results obtained in the study. The results suggested that crude leaf extract of *LP* has central and peripheral analgesic properties.

Key words: Antinocieptive · *Labisia Pumila* · Hot Plate · Formalin · Phytochemical

INTRODUCTION

Pain has been a major concern of humankind and has been the most frequent reasons that people seek medical attention. It is a complicated process that involves a complex interplay between a number of important chemicals found naturally in the brain and spinal cord. In general, these chemicals are called neurotransmitters and they transmit nerve impulses from one cell to another. There are many different neurotransmitters in the human body; some play a role in human disease and some in the case of pain, act in various combinations to produce painful sensations in the body

Apart from the body's natural painkiller, the treatment of pain requires analgesics including inflammatory products [1,2]. However it is believed that current analgesia inducing drugs such as opiates and NSAIDs are not useful in all cases, because of their side effects and potency. As a result, the search for other alternatives seems necessary and beneficial. Therefore, medicinal plants have been used in the development of new drugs and continue to play a vital role in the progress of drug discovery in this modern world. In this study, *Labisia pumila (LP)* plant was used as this plant has been claimed by the traditional practitioners as a pain reliever. Thus this claim has indeed initiated extensive research to be carried out on *LP* to verify the accurateness of it's claimed as antinociceptive properties and to determine its potential benefit to humankind in the treatment.

MATERIALS AND METHODS

Materials: The leaves of *LP* (Kacip Fatimah) were gotten from forest in Sungai Perak. The plant was specifically identified by Dr. Shamsul Khamis, a research officer (plant taxonomy) from the Laboratory of Natural Products (NATPRO), Institute of Bioscience in University Putra Malaysia.

Preparation of Plant Extracts: The leaves were air-dried for almost three weeks and were then grounded into fine powder using a miller. An extraction with MeOH and DCM was carried out by successive maceration at room temperature for a week followed by filtration. The filtration process was

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repeated several times to make sure all the dirt and dusts are completely removed [3]. The filtrate obtained after filtration was then concentrated by evaporation using a rotary evaporator at temperatures of 35□ until dryness to maximize the proportion of desired bioactive fractions contained in the solvents [4]. The process of extraction, filtration and concentration was repeated several times until there were maximum yield of crude MeOH and DCM extracts.

Phytochemical Analysis: Simple chemical test were performed to identify the possible bioactive fractions present in the effective fraction of the *LP*. The bioactive fractions that were tested for are flavonoids, alkaloids, tannins, steroids and saponins.

Animals: Healthy young adult Sprague-Dawley rats of both sexes weighing 200±SD g that were purchased from IMR were used in this study. The rats were screened and housed in standard polypropylene cages (three rats per cage), maintained under standard laboratory conditions (*i.e.* 12:12 hour light and dark cycle; at an ambient temperature of 25±5°C; 50-70 % of relative humidity); the animals were fed with standard rat pellet diet and water was made available at all times.

Acute Toxicity Studies: The acute toxicity study of the MeOH and DCM was performed in rats (n=10). In this assay, increasing concentrations of MeOH and DCM were orally administered to groups of animals for each dose after a 12h fast. Animals receiving the vehicle (saline) served as control. The signs and symptoms associated with the MeOH and DCM administration (5g/kg b. wt., per os/orally in 10mL) were observed at 0, 30, 60,120,180 and 240 min after administration and then once a day for the next 14days. At the end of the period the number of survivor was recorded. The acute toxicological effect was estimated by the method described by Souza and de Ensaios [5], the death, when occurred, was expressed as LD_{50} according to Litchfield and Wilcoxon [6].

Plant Extract and Drugs Treatments: All extracts of MeOH and DCM (50, 100 and 300 mg/kg each) was suspended in vehicle [2% of tragacanth powder, 2 drops of glycerol and Tween®40 in saline solution for rats, respectively]. Morphine sulphate (10mg/kg b.wt) dissolved in saline solution was used as antinociceptive reference drug. Drugs were freshly prepared on the day of the experiment.

Hot Plate Test: In this method, a 24 cm diameter glass cylinder was placed on a hot plate with temperature set at 55 ± 0.5 °C. Latency of the rats was determined before and after the treatment. The latency was recorded at the time before and 15, 30, 60, 75, 90 and 105 minutes after intraperitoneal administration of the extracts or drugs [7]. Each rat was placed on the hot plate in order to obtained the animals response to heat-induce antinociceptive pain stimulus. Response was defined as licking, or biting of the paw, or jumping where all four paws leave the plate. Time taken for each response was noted and recorded in seconds [8]. A latency period of 30s was fixed as the cut off time to prevent tissue damage to the rats [9] Eight group of rats (n=5) received MeOH and DCM (50, 100 and 300mg/kg each), 0.9% saline (1ml/kg) and morphine (10mg/kg) each.

Formalin Test: The method used was similar to what has been described by Hunskaar and Hole [9]. To induce nociception, rats were injected with 50 µl of 2.5% formalin in 0.9 % of saline solution into the subplantar surface of the left hind paw, 30 minutes after the administration of 0.9 % saline, aspirin (100mg/kg b.wt.) and plant extracts of different concentrations. Rats were then observed for 30 minutes and the time spent licking the paw was recorded in two phases. The data were express as total licking time in the early phase (0-5 min) and the late phase (15-30min) after formalin injection.

Statistical Analysis: The antinociceptive data were expressed as mean values ± standard deviation. Statistically significant differences between groups were measured using one-way analysis of variance (ANOVA) followed by Dunnett's test using statistical package for social science (SPSS 17th version) computer program. Values of *$p<0.05$, **$p<0.01$ and ***$p<0.001$ was considered statistically significant.

RESULTS AND DISSCUSSION

Oral administration of MeOH and DCM leaf extract at the concentration of 5000mg/kg b.wt., did not show any signs and symptoms of acute toxicity in all treated rats. No significant difference was observed in the weight of heart, liver, kidney, or lungs when they were compared with those of control group (saline). None of the treated rats died during the 14 days of observation after the administration of MeOH and DCM plant extract. The results obtained indicated the absence of acute toxic effect of MeOH and DCM.

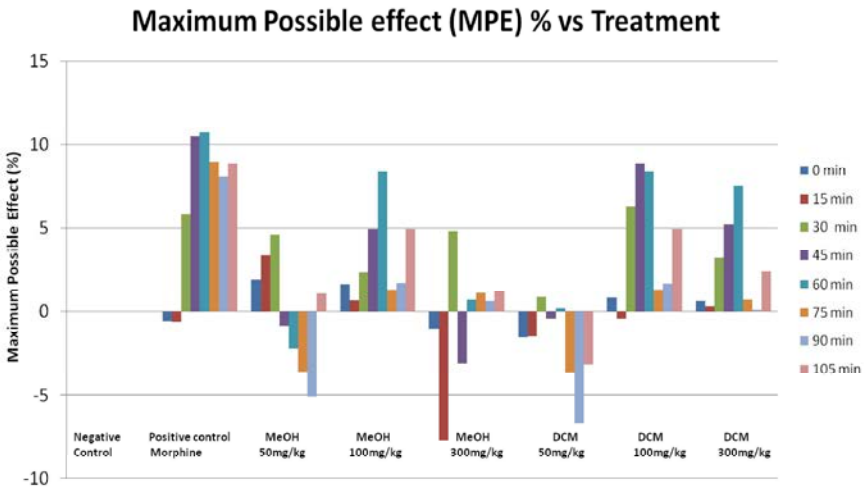

Fig. 1: Effect of Crude LP extracts on the latency time of rat submitted to the hot plate test and Maximum Possible Effect (MPE) % versus treatment

Table 1: Effect of Hot plate test on rats and its Maximum Possible Effect (MPE) % versus treatment

		Time(min) (MPE %)							
Treatment	Dose (mg/kg)	0	15	30	45	60	75	90	105
Negative control (Saline)	-	4.46±.319	4.80±1.15	4.58±1.16	5.62±0.20	4.80±0.84	5.26±1.08	5.07±1.45	4.04±0.69
Positive control(Morphine)	10mg/kg	4.32±0.48	4.65±0.88^{ns}	6.06±0.42^{ns}	8.18±0.9***	7.51±0.58**	7.48±0.58***	7.08±0.54^{n}	6.34±0.39***
		(-0.55)	(-0.60)	(5.82)	(10.50)	(10.75)	(8.97)	(8.06)	(8.90)
Methanol extract	50mg/kg	4.95±0.26	5.65±0.90^{ns}	5.75±0.96^{ns}	5.41±0.76^{ns}	4.24±0.37^{ns}	4.37±0.30^{ns}	3.80±0.53^{ns}	4.32±0.77^{ns}
		(1.92)	(3.37)	(4.60)	(-0.86)	(-2.22)	(-3.60)	(-5.09)	(1.08)
	100mg/kg	4.88±0.44**	4.97±1.22^{ns}	5.18±0.84^{ns}	6.83±0.79*	6.91±0.71***	5.58±1.37^{ns}	5.50±1.37^{ns}	5.32±0.17***
		(1.64)	(0.67)	(2.36)	(4.96)	(8.37)	(1.29)	(1.72)	(4.93)
	300mg/kg	4.20±0.80	4.43±0.45^{ns}	4.80±1.00^{ns}	4.87±0.55^{ns}	4.98±0.50^{ns}	5.46±0.66^{ns}	5.23±2.02^{ns}	4.35±0.15^{ns}
		(-1.01)	(-7.71)	(4.8)	(-3.08)	(0.71)	(1.13)	(0.64)	(1.19)
Dichloromethane extract	50mg/kg	4.07±0.37	4.30±1.30^{ns}	4.85±0.54^{ns}	5.52±0.35^{ns}	4.85±0.41^{ns}	4.36±0.24^{ns}	3.40±0.38^{ns}	3.23±0.15*
		(-1.53)	(-1.47)	(0.87)	(-0.41)	(0.20)	(-3.64)	(-6.70)	(-3.12)
	100mg/kg	4.68±0.44	4.69±1.22^{ns}	6.18^{ns}	7.83±0.79***	6.91±0.71***	5.58±1.37^{ns}	5.49±1.08^{ns}	5.32±0.17***
		(0.86)	(-0.44)	(6.29)	(8.90)	(8.37)	(1.29)	(1.68)	(4.93)
	300mg/kg	4.62±0.80	4.88±0.45^{ns}	5.40±1.00^{ns}	6.90±0.55*	6.70±0.50***	5.44±0.66^{ns}	5.10±2.02^{ns}	4.66±0.21^{ns}
		(0.62)	(0.32)	(3.21)	(5.25)	(7.54)	(0.73)	(0.12)	(2.39)

Values given with respect to the mean±SD, n=5 rats. Asterisks indicated significant difference from control. *p<0.05, **p<0.01, ***p<0.001 (ANOVA followed by Dunnett's test). MPE were calculated as percentage (%).

Table 2: Effect of Formalin test on rats and its Percentage of Inhibition

		Number of licking			
Group	Dose (mg/kg)	Early Phase (0-5min)	% inhibition	Late Phase	% inhibition (15-30)
Negative control (Saline)	-	64.67±6.04	0.00	30.83±2.83	0.00
Positive control(Aspirin)	100mg/kg	23±2.41***	75.17	0.00±0.00***	100.00
Methanol Extract	50mg/kg	49.00±6.50***	27.32	22.00±1.18***	28.64
	100mg/kg	37.10±0.53***	42.77	17.50±1.20***	43.23
	300mg/kg	28.60±1.38***	55.78	12.40±1.07***	59.78
Dichloromethane extract	50mg/kg	31.06±0.53***	51.97	21.33±0.50***	30.81
	100mg/kg	20.43±0.87***	68.41	15.67±0.65***	49.17
	300mg/kg	12.56±0.96***	80.58	7.86±0.61***	74.51

30 minutes after test drug administration (p.o), 2.5% formalin was subcutaneously injected to a hindpaw in volume of 50µl. Each data represent the mean number of licking time ± SD from 5 rats in the early phase (0-5 min) and late phase (15-30 min) after formalin injection. *p<0.05, **p<0.01, ***p<0.001 compared with the control group

In this experiment the hot plate provided the heat (thermal stimulus) and reactions such as licking the paws or jumping was observed. According to Mino *et al.*[10], the nociceptive response by the rats due to the hot plate was because of the direct activation of nociceptors (such as the C-fibers) in the central located dorsal horn. The tissue injury stimulates the central mediators such as substance P prostaglandins, CGRP, NGF and bradykinin [11,12].

Oral administration of MeOH and DCM crude extracts showed a significant (P<0.01) increase in the maximum possible effect (MPE) compared to negative control. The effect is comparable to that of the reference drug Morphine (10mg/kg), refer to table 1 and figure 1. The effect observed by the crude extract of LP may be as a result of its ability to deactivate the C- fibers in the central located dorsal horn or inhibition of the release of prostaglandins and glutamate and neuropeptides (especially substance P) from the primary afferent neuron or both.

The injection of 50 µl of 2.5% formalin produced the characteristic biphasic pain related behaviours (such as licking, flinching and biting of the injured paw [13]. The early phase (0-5min) appeared immediately following the formalin injection lasting for few minutes, corresponding to acute neurogenic pain, sensitive to drugs that interact with opioids system and the late phase (15-30 min) corresponding to inflammatory pain responses inhibited by analgesic – anti-inflammatory drugs [10]. Nociceptive behaviour evoked by formalin injection is associated with local inflammatory mediators such as prostaglandins, histamine and serotonin released at the site of tissue injury, sensitized or directly activated nociceptors[10,14].

In this experiment pre-treatment of the experimental rats with crude MeOH and DCM *LP* extract showed a marked reduction of the licking time in the early and late phase of formalin injection, respectively refer to table 2.

The result clearly shows that the extract may be acting by inhibition of the activation of C- fibers due to peripheral stimulus and inhibition of release of local inflammatory mediators such as prostaglandins, histamine and serotonin which causes functional changes in the dorsal horn of spinal cord [15,16].

Phytochemical screening of the extract showed the presence of flavonoids, saponins, terpenoids, steroids and tannins which have been reported to possess some antinociceptive effect.

CONCLUSION

In conclusion, the result of this research shows that extracts possess a concentration-dependent antinociceptive property. The effect may be due the presence of the phytochemicals. Further investigation are therefore required to isolated the actual bioactive compound present in the extract and elucidate its pharmacological mechanism of action.

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