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Antinociceptive and Anti-Inflammatory Effects of *Sida rhombifolia* L. in Various Animal Models

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Abstract: The present study was carried out to determine the antinociceptive and anti-inflammatory activities of the ethanolic extract of *Sida rhombifolia* L. leaves in various animal models. The antinociceptive activity was measured using the abdominal constriction test and hot plate test, while the anti-inflammatory was assessed using the carrageenan-induced paw edema test. The dried leaves of *S. rhombifolia* was mixed with 95% ethanol (1: 10; w/v), incubated in water bath (55°C) for 72 h, filtered and evaporated to dryness (50°C) under reduced pressure. The sticky paste of extract was then emulsified using 0.1% Tween-80 in normal saline at concentrations required to produce doses of 30, 100 and 300 mg kg^{-1} body weight. Based on the data obtained, all doses of the extract, administered intraperitoneally, exhibited significant ($p<0.05$) antinociceptive and anti-inflammatory activities in a dose-dependent manner. In conclusion, the leaves of *S.rhombifolia* possessed antinociceptive and anti-inflammatory activities and confirms its traditional uses in the treatment of pain-and inflammatory-related ailments.

Key words: *Sida rhombifolia*, ethanolic extract, antinociceptive activity, anti-inflammatory activity

INTRODUCTION

Sida rhombifolia L. is a plant that belongs to the family *Malvaceae* and is 1 of the medicinally important species of the genus *Sida*. *S. rhombifolia*, well known to the Malays as '*Ketumbar hutan*', is distributed widely throughout the world, including Malaysia, India, Puerto Rico etc. (Burkill, 1966). It is widely used in the traditional medicine practices of different cultures to treat various types of ailments like malaria, chest pain, rheumatism, fever, swellings, headache and abdominal pain (Burkill, 1966; Wilcox and Bodeker, 2004; Kulip, 1996; Acharya and Rai, 2006).

Scientific studies have revealed the presence of alkaloids (β-phenethylamines, quinazolines, carboxylated tryptamines, choline and betaine) (Praksh *et al*., 1981) and ecdysteroids (ecdysone, 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone-3-O-β-D-glucopyranoside and 20-hydroxyecdysone-3-O-β-D-glucopyranoside) (Jadhav *et al*., 2007) in this plant. Furthermore, *S. rhombifolia* was also found to exhibit *in vitro* antioxidant (Dhalwal *et al*., 2007), anti-inflammatory (Dunstan *et al*., 1997) and cytotoxic and antibacterial activities (Islam *et al*., 2003). Based on the traditional uses of this plant to treat and cure some diseases related to pain and inflammation, it is believed that *S. rhombifolia* possessed the antinociceptive and anti-inflammatory activities. Thus, the present study was performed to determine the antinociceptive and anti-inflammatory activities of the ethanolic extract of *S. rhombifolia* leaves (ESRL).

MATERIALS AND METHODS

Plant material and preparation of its ethanolic extract: The fresh leaves of *S. rhombifolia* were collected from the area of Sungkai, Perak, Malaysia and were identified by Mr. Shamsul Khamis, a botanist in the Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

The leaves of *S. rhombifolia* were cleaned by rinsing them with distilled water and then dried in an oven at 45°C for 1 week. The dried leaves were then ground into powder form, soaked in 95% ethanol in 1: 10 w/v ratio and then incubated (55°C) in water bath for 3 days followed

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by the filtration process (Whatman No. 1 filter paper). The collected supernatant was then evaporated to dryness at 50°C under reduced pressure and the dried extract obtain, labelled as ESRL, was kept at 4°C until used. Immediately before use, the ESRL was emulsified using 0.1% Tween-80 in normal saline at concentrations required to produce doses of 30, 100 and 300 mg kg^{-1} body weight for administration into the animals.

Preparation of drugs: 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, Universiti 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 (intraperitoneal; i.p.) dH_2O, 100 mg kg^{-1} ASA or 5 mg kg^{-1} morphine, or ESRL (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 6 groups of 6 rats each (n=6) for the anti-inflammatory study and received (*i.p.*) dH_2O, 100 mg kg^{-1} ASA or ESRL (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

Writhing test: The acetic acid-induced writhing test which has been slightly modified (Sulaiman *et al*., 2004) was used to evaluate the chemically-induced antinociceptive activity of ESRL.

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 ESRL.

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 ESRL.

Statistical analysis: The results are presented as Mean ± Standard Error of Mean (S.E.M.). 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 ESRL: The antinociceptive activity of ESRL assessed using the acetic acid-induced writhing test was shown in Table 1. The ESRL, at all doses used, significantly ($p<0.05$) reduced the number of writhing response in dose-dependent manner. The percentage of antinociception recorded was 67.93, 76.65 and 92.99%, respectively. The 30 mg kg^{-1} dose of ESRL produced an equal effective activity when compared to the 100 mg kg^{-1} ASA, indicated by their insignificant percentage of analgesia (76.65 vs. 80.96%).

The antinociceptive activity of ESRL assessed using the thermal-induced nociceptive test was shown in Table 2. All doses of ESRL significantly ($p<0.05$) increase the latency of discomfort at different interval time. The 10 mg kg^{-1} dose of ESRL produced an onset after 120 min of its administration and the activity lasted until the end of the experiments. The onset of action for the 30 and 100 mg kg^{-1} doses of ESRL occurred at the interval of 60 and 30 min, respectively, after their administration. Although, all doses of the extract antinociceptive activity lasted until the end of the experiment, their activity were remarkably lowered, at least at the first 2 h, than the reference drug (5 mg kg^{-1} morphine).

The anti-inflammatory profile of the ESRL: The ESRL, at all doses tested, exhibited significant ($p<0.05$) anti-inflammatory activity (Table 3). The activity was observed after 1 h or the extract administration and lasted until the end of the experiment.

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

Treatment groups (n=8)	Writhing response	Inhibition (%)
Saline	111.33 ± 10.51	-
100 mg kg^{-1} ASA	21.17 ± 4.32*	80.96
10 mg kg^{-1} ESRL	35.70 ± 5.41*#	67.93
30 mg kg^{-1} ESRL	26.00 ± 1.57*	76.65
100 mg kg^{-1} ESRL	2.3 ± 0.84*#	92.99

The writhing response was expressed as mean ± S.E.M.; *Data differs significantly ($p\leq0.05$) when compared against the normal saline-treated group; # Data differs significantly ($p\leq0.05$) when compared against ASA-treated group

Table 2: Antinociceptive activity of ESRL 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	5.95 ± 0.13	6.57 ± 0.32	6.50 ± 0.30	6.98 ± 0.23	6.48 ± 0.42
5 mg kg^{-1} morphine	5.73 ± 0.20	18.12 ± 3.32*	19.83 ± 2.99*	16.80 ± 1.48*	11.27 ± 1.16*
10 mg kg^{-1} ESRL	6.07 ± 0.28	8.05 ± 0.48§	7.57 ± 0.44§	9.15 ± 0.48§	11.27 ± 0.73*
30 mg kg^{-1} ESRL	6.20 ± 0.18	8.17 ± 0.38*§	9.58 ± 0.45*§	10.57 ± 0.23*	11.60 ± 0.45*
100 mg kg^{-1} ESRL	6.33 ± 0.14	10.67 ± 0.24*	10.43 ± 0.45*	10.95 ± 0.65*	12.10 ± 0.43*

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

Table 3: Anti-inflammatory activity ESRL 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	3 h	5 h	7 h	24 h
Saline	1.41 ± 0.02	1.60 ± 0.04	1.58 ± 0.03	1.40 ± 0.02	1.35 ± 0.02
100 mg kg^{-1} ASA	0.86 ± 0.04*	1.09 ± 0.09*	1.13 ± 0.06*	0.93 ± 0.06*	0.82 ± 0.03*
10 mg kg^{-1} ESRL	0.90 ± 0.09*	1.22 ± 0.07*	1.02 ± 0.09*	1.14 ± 0.04*	0.96 ± 0.07*
30 mg kg^{-1} ESRL	1.16 ± 0.05*	1.25 ± 0.05*	1.13 ± 0.05*	1.06 ± 0.06*	1.02 ± 0.06*
100 mg kg^{-1} ESRL	1.01 ± 0.05*	1.03 ± 0.05*	0.97 ± 0.06*	0.86 ± 0.06*	1.01 ± 0.04*

The volume of hind paw oedema was expressed as mean ± S.E.M; *Data differs significantly ($p \le 0.05$) when compared against the normal saline-treated group

DISCUSSION

The ESRL exhibited antinociceptive activity when assessed by the abdominal constriction test and hot plate test suggesting the involvement of peripheral and central mechanisms (Pini *et al*., 1997; Amanlou *et al*., 2005). The ability to inhibit both the chemically-and thermally-induced nociception indicate that the extract possessed a strong analgesics characteristic (Hosseinzadeh and Younesi, 2002).

The extract's anti-inflammatory activity assessed using the carrageenan-induced paw edema assay suggested the involvement of peripheral cyclo-oxygenase (COX) (Deraedt *et al*., 1980; Berkenkopf and Weichman, 1988), which is backed by Gamache and Povlishock (1986) report that the assay is more effectively blocked by the inhibitors of arachidonate COX, but not the inhibitors of arachidonate LOX. In addition anderson Dunstan *et al*. (1997) have also reported on *S. rhombifolia* anti-inflammatory activity assessed using the rat ear edema test that involved the modulation of prostaglandin biosynthesis.

The antinociceptive activity of ESRL is believed to involve, partly, the blockade of the peripheral cyclo-oxygenase (COX) or prostaglandin synthesis/action. The ability of ESRL to inhibit the writhing response, which involve the release of PGE_2 and $PGE_{2\alpha}$ (Vogel and Vogel, 1997) and COX-synthesized prostacyclin (Ballou *et al*., 2000) could be used to support our suggestion. The presence of steroids in the *S. rhombifolia* could also be linked to the observed antinociceptive activity based on previous report that steroids from other plant also exert the same activity (Meotti *et al*., 2005).

CONCLUSION

In conclusion, the present study demonstrated the potential of *S. rhombifolia* 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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