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Antinociceptive and Anti-Inflammatory Activities of the Chloroform Extract of *Bauhinia purpurea* L. (Leguminosae) Leaves in Animal Models

[1]Z.A. Zakaria, [2]N.I. Abdul Rahman, [2]Y.W. Loo, [2]A.H. Abdul Ayub,
[3]M.R. Sulaiman, [3]A.M. Mat Jais, [1]H.K. Gopalan and [2]C.A. Fatimah
[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, 40450 Shah Alam, Selangor, Malaysia
[2]Faculty of Biotechnology and Life Sciences, Industri Selangor University,
Jalan Zirkon A7/A, Seksyen 7, 40000 Shah Alam, Selangor, Malaysia
[3]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences,
Putra Malaysia University, 43400 Serdang, Selangor, Malaysia

Abstract: *Bauhinia purpurea* L. (Leguminosae), has been used traditionally to treat ailments like sores, wounds and diarrhea. The present study was carried out to establish the antinociceptive and anti-inflammatory activities of chloroform extract of *B. purpurea* leaves using animal models. The air-dried, powdered leaves (≈ 20 g) were soaked in chloroform (1:20; w v^{-1}) for 72 h and the supernatant obtained was then evaporated to dryness. The crude dried extract (≈ 1.323 g), dissolved in dimethyl sulfoxide, was prepared in the doses of 20, 100 and 200 mg kg^{-1} and then subjected to the respective antinociceptive (abdominal constriction, hot plate and formalin tests) and anti-inflammatory (carrageenan-induced paw edema test) assays. The results obtained indicate that the extract possess significant ($p<0.05$), but concentration-independent, antinociceptive activity when assessed using the abdominal constriction-and hot plate-test. This activity was also, significantly ($p<0.05$) observed in the early and late phases of the formalin test. The extract also exhibited significant ($p<0.05$) anti-inflammatory activity in a non-concentration-dependent manner. Unexpectedly, the 100 mg kg^{-1} extract showed a less remarkable anti-inflammatory activity compared to the other doses tested. The chloroform extract of *B. purpurea* contain bioactive compounds with potential peripheral and central antinociceptive and anti-inflammatory activities that requires further investigation.

Key words: *Bauhinia purpurea*, chloroform extract, antinociceptive, anti-inflammatory, dose-independent

INTRODUCTION

Bauhinia purpurea L. (family Leguminosae), known to the Malays as *pokok tapak kerbau*, has been traditionally used by the Indian, Sri Lankan and Pakistani peoples to treat ailments like ulcers, wounds, glandular swellings and stomach tumors (Lanka Chronicle, 2006). However, there is no documentation on its traditional uses to treat diseases among the locals in Malaysia. Scientifically, *B. purpurea* has been reported to be a potential antihyperglycemic agent (Salatino *et al*., 1999). The plant also has been claims to possess laxative and astringent effects (Lanka Chronicle, 2006) and in Pakistan, the flowers of *B. purpurea* have been used as purgative (Lanka Chronicle, 2006).

Earlier study on the bioactive compounds from *B. purpurea* was carried out by Bhartiya *et al.* (1979) and lead to the isolation of chalcone glycoside (butein 4'-O-β-arabino-O-β-galactoside). This is followed by isolation of 3,4-dihydroxychalcone 4-O-β--arabinopyranosyl-O-β-galactopyranoside, a glycoside from its seeds (Bhartiya and Gupta, 1999). Recent studies have demonstrated the lipid classes and compositions of fatty acids and fat-soluble bioactive compounds in the n-hexane extract of *B. purpurea* seed oil (Ramadana *et al*., 2006). The extract, which is high in neutral lipids, followed by glycolipids and phospholipids and was also found to contain high amount of linoleic acids, followed by palmitic, oleic and stearic acids. β-sitosterol, stigmasterol, β-tocopherol and d-tocopherol were also detected in the seed oil extract (Ramadana *et al*., 2006).

In spite of the traditional claims and identified bioactive compounds, no thorough scientific study has been carried out to establish the pharmacological properties of *B. purpurea* leaves. Thus, the objective of the present study was to verify the antinociceptive and anti-inflammatory activities of the chloroform extract of *B. purpurea* leaves using various animal models.

Corresponding Author: Zainul Amiruddin Zakaria, Department of Pharmaceutical Sciences, Faculty of Pharmacy, 40450 Shah Alam, Selangor, Malaysia

MATERIALS AND METHODS

Plant material: The leaves of *B. purpurea*, collected from its natural habitat in Shah Alam, Selangor, Malaysia, were sent to the Institute of Bioscience (IBS), University Putra Malaysia (UPM), Serdang, Selangor, Malaysia for the purpose of species identification. A voucher specimen (SK 1095/05) was deposited at the Herbarium of the Laboratory of Natural Products, IBS, UPM, Serdang, Selangor, Malaysia.

Phytochemical screening of the *B. Purpurea* leaves: The leaves of *B. purpurea* were subjected to the phytochemical screening (Ikhiri *et al.*, 1992) to determine the presence of flavonoids, triterpenes, tannins, alkaloids, saponins and steroids.

Preparation of Chloroform Extract of *B. purpurea* (CEBP): The CEBP was prepared by soaking the air-dried powdered leaves of *S. nigrum* (20 mg) in chloroform in the ratio of 1:20 (w v^{-1}) for 72 h. The supernatant was collected and filtered using Whatman No. 1 filter paper while, the remaining plant residue was discarded. The filtered supernatant obtained was evaporated to dryness and the weight of the crude dried chloroform extract obtained was measured (approximately, 1.323 g). The dried extract was then diluted in Dimethyl Sulfoxide (DMSO) (1:50; w v^{-1}) and considered as the stock solution with dose of 200 mg kg^{-1}. The stock solution was then diluted with DMSO to the doses of 20 and 100 mg kg^{-1} for antinociceptive and anti-inflammatory studies.

Preparation of drugs solutions: Acetylsalicylic acid (ASA) (Bayer, Singapore) and morphine (Sigma, Germany), prepared in the doses of 100 and 5 mg kg^{-1} by dissolving them in distilled water (DH_2O), respectively, were used as positive control drugs.

Experimental animals: Male Balb-C mice (25-30 g; 5-7 weeks) and Sprague-Dawley rats (180-200 g; 8-10 weeks old) were obtained from the Institute of Medical Research, Kuala Lumpur, Malaysia. The animals were kept under room temperature (27±2°C; 70-80% humidity; 12 h light/darkness cycle) for at least 48 h before use in the Animal Holding Unit, Faculty of Medical and Health Sciences, UPM. Food and water were supplied *ad libitum* up to the beginning of the experiments. The animals were cared for throughout the experimental time according to the UPM ethical guidelines for investigations of experimental pain in conscious animals adopted from Zimmermann (1983).

Ten groups of equally divided mice (n = 7) were subcutaneously administered with DH_2O, ASA (100 mg kg^{-1}) or CEBP (20, 100 and 200 mg kg^{-1}) 30 min prior to subjection to the abdominal constriction or hot plate tests, respectively. Eleven groups of equally divided rats (n = 5), on the other hand, were used in the formalin and anti-inflammatory tests. The first six groups, used in the formalin test, received (sc) DH_2O, 100 mg kg^{-1} ASA, 5 mg kg^{-1} morphine or CEBP (20, 100 and 200 mg kg^{-1}), respectively 30 min prior to subjection to the test while the other five groups, which received (sc) the respective DH_2O, 100 mg kg^{-1} ASA or CEBP (20, 100 and 200 mg kg^{-1}), were subjected to the carrageenan-induced paw edema test 30 min later. All of the test solutions were administered in the volume of 10 mL kg^{-1} body weight.

Antinociceptive assays

Abdominal constriction test: The abdominal constriction test with slight modifications was used to evaluate the chemically-induced antinociceptive activity of AEBP as described in detail by Zakaria *et al.* (2005).

Hot plate test: The 50°C hot-plate test was used with slight modifications as described by Zakaria *et al.* (2005) to assess the thermal-induced central antinociceptive activity of AEBP.

Formalin test: The formalin test was used with slight modifications as described by Zakaria *et al.* (2006) to assess the antinociceptive, non-anti-inflammatory properties of the AEBP.

Anti-inflammatory assay: The carrageenan-induced paw edema test was used with slight modifications as described by Zakaria *et al.* (2006) to assess the anti-inflammatory effect of the AEBP.

Statistical analysis: The results are presented as Mean±Standard Error of Mean (SEM). The one-way ANOVA test with Tukey post-hoc test was used to analyze and compare the data obtained for the abdominal constriction and formalin assays while, the two-way ANOVA test with Tukey post-hoc test was used to analyze and compare the data obtained for the hot plate and paw edema assays. $p<0.05$ was used as the limit of significance.

RESULTS

Phytochemical screening of the *B. purpurea* leaves: The phytochemical screening of *B. purpurea* leaves has demonstrated the present of flavonoids, saponins, triterpenes and steroids, but no alkaloids and tannins (Table 1).

Table 1: Phytochemical constituents of *B. purpurea*

Chemical constituents	*B. purpurea*
Flavonoids	+
Triterpenes	+
Tannins	-
Alkaloids	-
Saponins	++
Steroids	+++

For saponins: (+: 1-2 cm froth; ++: 2-3 cm froth; +++: –>3 cm froth), For flavonoids, tannins, triterpenes and steroids: (+: Weak colour; ++: Mild colour; +++: Strong colour), For akalioids, (+: Negligible amount of precipitate; ++: Weak precipitate; +++: Strong precipitate)

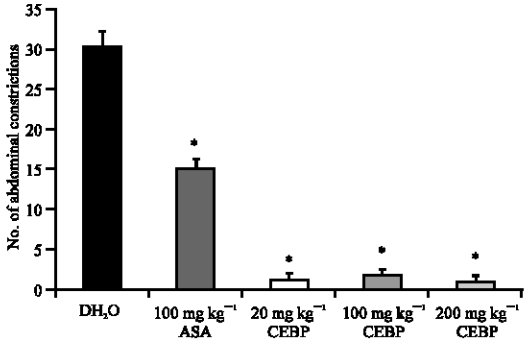


Fig. 1: The antinociceptive effect of CEBP assessed by the abdominal constriction test in mice (Data differ significantly ($p<0.05$) when compared against the DH_2O-treated group)

Pharmacological studies on the CEBP: The CEBP was found to produce significant ($p<0.05$) reduction in the number of abdominal constrictions at all doses tested (Fig. 1). In term of the percentage of antinociception, all doses of CEBP gave above 90% antinociceptive activity compared to the 100 mg kg^{-1} ASA that gave approximately, 50% activity.

The CEBP antinociceptive activity was found to occur in a concentration-independent manner when assessed using the hot plate test in mice (Fig. 2). The 20 and 50 mg kg^{-1} CEBP exhibited their significant ($p<0.05$) activity at the interval of 4-5 and 3-5 h after administration of test solutions. The 100 mg kg^{-1} CEBP, on the other hand, exhibited a significant ($p<0.05$) activity after 2 h of its administration into mice and this activity lasted for another 3 h (interval 5 h) before completely diminished at the end of the experiment. Interestingly, the activity of the three doses of CEBP used was insignificant when compared to that of 5 mg kg^{-1} morphine at the interval time of 5 h indicating their similar effectiveness at the said time. Overall, morphine activity was greater than the extract.

The antinociceptive activity of CEBP assessed using the formalin test was found to occur in a concentration-indeendent manner (Fig. 3). The extract, at all doses tested, exhibited significant ($p<0.05$) antinociceptive activity in the early and late phases of the test.

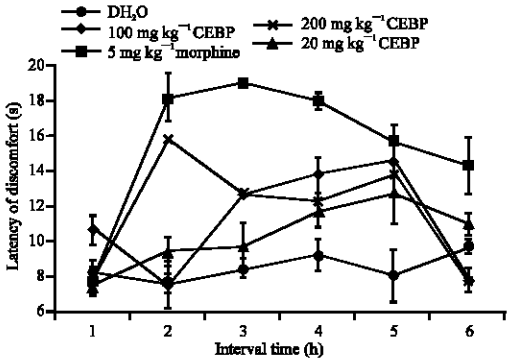


Fig. 2: The antinociceptive effect of CEBP assessed by the hot plate test in mice (Data differ significantly ($p<0.05$) when compared against the DH_2O-treated group)

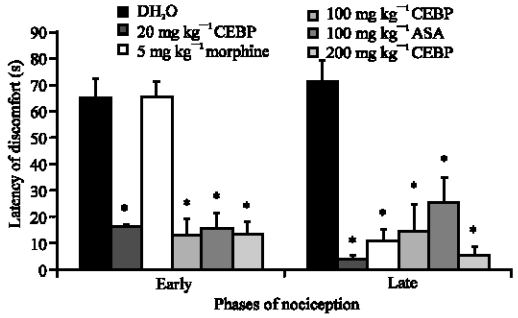


Fig. 3: The antinociceptive effect of CEBP assessed by the formalin test in rats

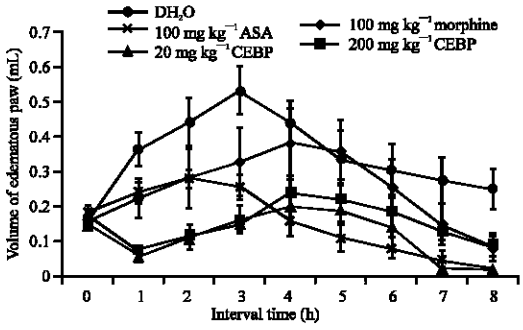


Fig. 4: The anti-inflammatory effect of CEBP assessed by the carrageenan-induced paw edema test in rats

Interestingly, all doses of CEBP, which produced an activity that are insignificant when compared against each other, exhibited an activity that is comparable to 5 mg kg^{-1} morphine in the early phase. The activity of CEBP in the late phase was comparable to both 5 mg kg^{-1} morphine and 100 mg kg^{-1} ASA.

The anti-inflammatory activity of CEBP, assessed using the carrageenan-induced paw edema test in rats, occurs in a non-concentration dependent manner (Fig. 4).

Inflammation induced by carrageenan was found to reach its peak after 3 h of its administration and gradually decreased until the end of the experiment. Unexpectedly, the 100 mg kg^{-1} CEBP produced a less remarkable activity with significant ($p<0.05$) activity observed only at the interval of 1 h. Interestingly, the 20 and 200 mg kg^{-1} CEBP were found to exhibit significant ($p<0.05$) anti-inflammatory activity in a similar degree indicated by their insignificant effect when compared against each other. This activity was observed at the interval of 1 h and lasted for another 5 h before completely diminished. The 100 mg kg^{-1} ASA, on the other hand, produced significant ($p<0.05$) activity after 3 h of its administration and this activity lasted until the end of the experiment.

DISCUSSION

B. purpurea has been used traditionally by peoples in India, Sri Lanka and Pakistan to treat ailments related to pain and inflammation. The present study has scientifically shown the potential of *B. purpurea* as antinociceptive and anti-inflammatory agents when tested against various animal models. In term of mechanism, the ability of CEBP to inhibit the abdominal constriction, hot plate and formalin tests indicates the extract ability to inhibit chemically-and thermally-induced noxious stimuli. Furthermore, the extract was also believed to modulate the antinociceptive activity via peripheral and central mechanism (Hosseinzadeh and Younesi, 2002; Chan *et al*., 1995). In addition, the CEBP ability to inhibit both types of stimuli (Hunskaar *et al*., 1986) and to inhibit both phases of the formalin test (Pini *et al*., 1997) suggested that the extract possesses a characteristic of strong analgesic. Based on claims made by Ballou *et al*. (2000) and Pini *et al*. (1997), the ability of CEBP to inhibit/reverse abdominal constriction and hot plate tests, together with its ability to affect both phases of the formalin test, indicate the extract ability to inhibit/reverse the inflammatory-induced and non-inflammatory-related nociception, as well as to produce an antinociceptive, non-anti-inflammatory effect (Hunskaar *et al*., 1985).

The abdominal constriction response involved the release of peripheral prostaglandins and the ability of the extract to inhibit/reverse the test could be linked to the inhibition of peripheral COX activity (Vogel and Vogel, 1997) while, the central antinociceptive mechanism seen with the hot plate test have also been related, in part, to inhibition of the central COX activity (Uzcategui *et al*., 2004). Furthermore, although not yet proven, the involvement of Nitric Oxide/cyclic Guanosine Monophosphate (NO/cGMP) pathway in the antinociceptive activity of CEBP could also be suggested based on reports that some Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) also produced an antinociceptive activity via modulation of the NO/cGMP pathway (Granados-Soto *et al*., 1995). As described in the above paragraph, the CEBP was found to show a characteristic of strong analgesic, which could then be associated, in part, with the opioids-like mechanism (Hosseinzadeh and Younesi, 2002; Chan *et al*., 1995). On the other hand, Patil *et al*. (2003) have also linked the inhibition of COX activity during the antinociceptive mechanism with the activation of the NO/cGMP pathway with the latter also associated with the modulation of the centrally acting drugs (i.e. opioid) activity.

According to Brooks and Day (1991), the carrageenan-induced inflammatory process have been shown to be a COX-dependent response and is more effectively controlled with arachidonate COX, but not arachidonate lipo-oxygenase, inhibitors.

Phytochemical screening of the leaves of *B. purpurea* has demonstrated the presence of flavonoids, saponins, triterpenes and steroids. Flavonoids, like kaempferol, quercetin and isorhamnetin have been isolated form the leaves of *B. purpurea* (Salatino *et al*., 1999). In addition, Pettit *et al*. (2006) have successfully isolated pacharin and bauhiniastatins from various parts of *B. purpurea*, including its leaves. Compounds like flavonoids (Kim *et al*., 2004) and triterpenes (Beirith *et al*., 1999) have been demonstrated to show antinociceptive and anti-inflammatory activities, which is in line with claim made by Attaway and Zaborsky (1993) that compounds with anti-inflammatory activity also possess antinociceptive activity and thus supported the findings on CEBP pharmacological activities.

Several mechanisms of action could be hypothesized to explain the pharmacological activities seen with CEBP based on the classes of compounds detected in it. Olszanecki *et al*. (2002) have demonstrated that flavonoids are potent inhibitors of nitric oxide synthase type 2 and protein tyrosine kinases, both of which are important enzymes that take part in a series of molecular events within the NO/cGMP pathway. Earlier, Robak *et al*. (1998) have reported on the ability of flavonoids to stimulate NOS-2 via indirect inhibition of the COX and/or lipoxygenase pathways. Other than that the flavonoids (Meotti *et al*., 2005) and triterpenes (Otuki *et al*., 2005) antinociceptive activity have also been reported to involve inhibition of the protein kinase C and/or L-arginine/NO pathways. In addition, Rajendran *et al*. (2000) and Otuki *et al*. (2005) have reported that flavonoids', but not triterpenes', antinociception involved modulation of the opioid receptor. Meanwhile, Middleton *et al*. (2000) have shown the ability of flavonoids to block

phospholipase A2 and phospholipase C, which are key enzymes in the inflammatory processes. The ability of flavonoids and triterpenes to inhibit the nuclear factor-kappaB (NF-κB) could be suggested to contribute to the CEBP's anti-inflammatory activity (Nam, 2006).

CONCLUSION

The present study demonstrated that the CEBP possessed antinociceptive and anti-inflammatory, which merit further investigation. The observed activities could be due to synergistic activity of the bioactive compounds, particularly flavonoids, triterpenes, saponins and steroids that are presence in the leaves of *B. purpurea*. Finally, the present study confirms the folklore uses of the plant for the treatment of various ailments.

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