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Bioactivities of *Picrasma javanica* Blume. leaf extract

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***Picrasma javanica* Blume. is locally known as Nilghanta. Traditionally, the plant is used as an anti-pyretic. The aim of this study was to determine the biological properties of *P. javanica* leaf extract. The writhing test and tail flick response were determined to assess peripheral and central analgesic activities, respectively. Castor oil was fed orally to induce diarrhea in the test animals. Paw edema method was followed to determine the anti-inflammatory potential. Neuropharmacological property of the extract was determined using phenobarbitone sodium. 400 mg/kg body weight dose of *P. javanica* leaf extract inhibited 70.41% writhing which was comparable to that of diclofenac sodium (75.71% inhibition). The extract also reduced diarrheal feces by 63.10% and carrageenan induced rat paw edema by 91.49% at the same dose. On the other hand, *P. javanica* extract increased the duration of phenobarbitone sodium-induced sleep. *P. javanica* leaf extract is a suitable future candidate for phytochemical screening.**

Key words: Acetic acid, writhing, castor oil, paw edema, carrageenan.

INTRODUCTION

Among the nine species under *Picrasma* genus, three species (*P. quassioides*, *P. javanica* and *P. excelsa*) have been most extensively studied (Zhang et al., 2018). *Picrasma javanica* Blume. belongs to Simarubaceae family. Other synonyms include *Picrasma nepalensis* A.W. Bennet. and *Picrasma philippinensis* Elmer. Its Bengali name is Nilghanta. This medium sized tree can attain a height of 20 m. The plant can grow at an altitude of 150 to 1400 m. The presence of quassinoid type compounds contribute to the bitterness of the leaves, bark and fruits of the species (Hidayat, 2003). The plant grows abundantly in North-Eastern India, South-East Asia and the coastal areas of Bangladesh. In Myanmar, Thailand and Indonesia, bark of the plant is used to reduce fever (Heyne, 1987; Zhang et al., 2018). Quassinoids like javanicin A-Z; picrajavanin A-M (Koike and Ohmoto, 1990; Koike et al., 1990, 1991[a, b, c, d]; 1995; Win et al., 2015, 2016; Guo et al., 2005) and quassinoid glycosides like javanicinoside B-L (Koike et al., 1990; Koike and Ohmoto, 1992; Ishii et al., 1991) are found to be the major chemical constituents from *P. javanica*. 5-hydroxydehydrocrenatine, 5-hydroxycrenatine, 4-methoxy-1-vinyl-β-carboline, 6-hydroxy-4-methoxy-1-vinyl-β-carboline and javacarboline are some of the reported alkaloids from the species (Arbain et al., 1990; Johns et al., 1970; Pavanand et al., 1988; Koike et al., 1994). Although quassinoids are the well-known phytocomponents from the species, alkaloids were found

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to be responsible for the anti-malarial potential (Saiin, 2017; Pavanand et al., 1988; Saiin et al., 2016). The antioxidant, cytotoxic, thrombolytic, anti-malarial and anti-cancer activities of the leaf extract have been reported (Sharmin et al., 2012; Saiin et al., 2003).

This study was performed as part of the attempt to evaluate the medicinal plants of Bangladesh (Sharmin et al., 2018; Sharmin and Rashid, 2020). Herein, the results of analgesic, anti-diarrheal, anti-inflammatory activities along with sleep inducing potential assays using *P. javanica* leaf extract are reported.

MATERIALS AND METHODS

Plant

Collection of plant materials

Leaf of *P. javanica* was obtained from Khagrachori, Bangladesh. Salar Khan Herbarium, Department of Botany, University of Dhaka preserved a voucher specimen (DUSH–10771) for this collection.

Extraction and fractionation

After collection, the leaves were cleaned, chopped into smaller pieces and dried for several days. The dried leaves were powdered, and 500 g of the powdered material was soaked in 1.5 L methanol for seven days. After that, the macerated plant material was filtered, and the filtrate was concentrated using a rotary evaporator.

Test animals

For the determination of analgesic, anti-diarrheal and sleep-inducing activities, healthy Swiss-albino mice (age: 4-5 weeks, weight: 25-30 g) and for the assessment of anti-inflammatory activity, Wistar rats (weight: 150-200 g) were used. All the animals were supplied by the Animal Resources Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). Standard room temperature (25 ± 2°C), relative humidity (55-60%) and other living conditions like a 12 h light-dark cycle, adequate supply of food and water were maintained. The animals were nurtured for fifteen days to let them adapt with the laboratory environment (Hawk et al., 1954). Standard guidelines for using animals in scientific experiments were followed. All the experiments were conducted at State University of Bangladesh after taking approval from its ethics committee.

Drugs and chemicals

Gonoshastho Pharmaceuticals Ltd., Bangladesh and Incepta Pharmaceuticals Ltd., Bangladesh provided all the drugs required for the assays. The rest of the reagents and solvents were supplied by Sigma-Aldrich, Germany.

Peripheral analgesic activity assay by writhing method

In peripheral analgesic activity assay, the samples were evaluated by determining their ability to inhibit acetic acid induced abdominal writhing (Kaushik et al., 2012). Twenty mice were used in this assay and divided into four groups namely positive and negative control groups and sample groups. Distilled water (10 ml/kg) was fed orally to the mice of negative control group while diclofenac sodium (50 mg/kg body weight) was given orally to the positive control group. Sample groups received the plant extract (200 and 400 mg/kg doses). 1% v/v acetic acid (10 ml/kg dose) was given intraperitonially to all the test groups. After 10 min of administering acetic acid, the animals started to exhibit a stretch reflex due to the abdominal pain. This manifestation is called writhing. The number of abdominal writhing was recorded for each animal for the next 10 min. The pain inhibition percentage was determined using the following equation:

$$\text{Percentage inhibition} = \frac{[\text{Mean number of writhing (control)} - \text{Mean number of writhing (test)}] \times 100\%}{\text{Mean number of writhing (control)}}$$

Determination of central analgesic activity

The central analgesic activity was determined by immersing the tail tip of the animals into a radiant heat source. The tail withdrawal time was recorded (Pizziketti et al., 1985). Twenty mice were used in this assay and divided into four groups, namely positive control, negative control and sample groups. Distilled water (10 ml/kg) was fed orally to the mice of negative control group. Morphine (2 mg/kg body weight) was given subcutaneously to mice of positive control group. Treatment groups received the extract at 200 and 400 mg/kg doses. At 30, 60 and 90th min, the tail flick response time was recorded. The pain inhibition percentage was determined using the following equation:

Pain inhibition percentage = $((T_1-T_0)/T_0) \times 100\%$

where T_1 is post-drug latency and T_0 is pre-drug latency.

Anti-diarrheal activity by castor oil challenge

In this assay, 1 ml pure analytical grade castor oil was fed to the mice to induce diarrhea. The number of fecal stools excreted was recorded (Shoba and Thomas, 2001). Twenty mice were used in this assay and divided into four groups namely positive and negative control groups and sample groups. Distilled water (10 ml/kg) was fed orally to the mice of negative control group while loperamide (50 mg/kg) was given orally to mice of positive control group. The sample groups received the plant extract at 200 and 400 mg/kg body weight doses. 1 ml analytical grade castor oil was given to all the mice. The animals were placed in individual cages and the number of fecal discharges of individual mouse was recorded for next 4 h.

Evaluation of anti-inflammatory activity

In this assay, carrageenan was injected in the plantar surface of the right hind paw of Wistar rats. This resulted in an inflammatory response manifested by paw edema (Amdekar et al., 2012). Cotton thread method was used to measure paw circumference at 1st, 2nd, 3rd and 4th hour of carrageenan injection. In this assay, indomethacin (10 mg/kg) was used as the positive control. The following equation was used to calculate percent inhibition of paw edema:

% Paw edema inhibition = $[\{(C_t-C_o)\ \text{control}-(C_t-C_o)\ \text{treated}\} / (C_t-C_o)\ \text{control}] \times 100\%$

where C_o paw circumference at zero time (before carrageenan injection), C_t = paw circumference at time t, and (C_t-C_o) = paw edema.

Table 1. Effect of *P. javanica* leaf extract on acetic acid induced writhing in mice.

Group	Number of writhing*	Inhibition of writhing (%)
Control (10 ml/kg)	19.6	
Diclofenac sodium (Standard) (50 mg/kg)	5.00±0.08	75.71
Methanolic crude extract (200 mg/kg)	6.43±0.05**	67.35
Methanolic crude extract (400 mg/kg)	5.88±0.11**	70.41

Values are expressed as mean ± SD; **p<0.01.
Source: Authors 2022

Table 2. Effect of *P. javanica* extract on tail flicking time of mice.

Group	Latency period			
	0 min	30 min	60 min	90 min
Control (10 ml/kg)	6.03±0.17	6.26±0.36	6.40±0.03	6.00±0.45
Morphine (Standard) (2 mg/kg)	6.48±0.09	10.80±0.11**	15.30±0.12**	10.22±0.37**
Methanolic crude extract (200 mg/kg)	6.05±0.17	9.20±0.21*	7.74±0.02	6.14±0.04
Methanolic crude extract (400 mg/kg)	6.15±0.21	9.37±0.14*	8.52±0.17	7.02±0.08

Values are expressed as mean ± SD; *p<0.05, **p<0.01.
Source: Authors 2022

Phenobarbitone induced sleeping time test

In this assay, phenobarbitone sodium (25 mg/kg body weight) was given intraperitonially to all the test groups to induce sleep (Williamson et al*.,* 1996). Before that, mice of control group were fed with 1% Tween-80 in saline mixture (10 ml/kg body weight). The crude methanol extract (200 and 400 mg/kg body weight) was given orally to the test groups. All the animals were then observed for the time of onset of sleep and total duration of sleep.

Statistical analysis

Three independent experiments were performed to determine the average ± standard deviation (SD). A two tailed non-parametric student's t-test was used to calculate the P values.

RESULTS AND DISCUSSION

Traditionally, *P. javanica* extract is used in malaria in Myanmar, Thailand, Indonesia and Northern India (Saiin et al., 2017; Bora et al., 2007; Lalmuanpuii et al., 2013). This study was aimed to determine the biological activities of *P. javanica* leaf extract.

P. javanica leaf extract (400 mg/kg dose) inhibited acetic acid induced writhing by 70.41% at a dose of 400 mg/kg body weight which was comparable to the standard, diclofenac sodium (75.71%) (Table 1).

In central analgesic activity assay, *P. javanica* leaf extract elongated the reaction time by 46.96 and 49.68% after 30 min of administration of the extract at 200 and 400 mg/kg body weight doses, respectively (Tables 2 and 3).

P. javanica extract is rich in quassinoid type compounds (Alves et al., 2014). Therefore, it can be hypothesized that the bioactivities observed from this plant extract might be contributed by these compounds. *β*-sitosterol has been reported to be present in this species (Saiin et al., 2003). Different analgesic and anti-inflammatory assay models have already shown the efficiency of *β*-sitosterol as a potent analgesic and anti-inflammatory compound (Nirmal et al., 2012; Gupta et al., 1980).

Diarrheal feces were significantly lowered (63.10%) by *P. javanica* extract at a dose of 400 mg/kg body weight. The result was comparable to standard loperamide (71.42%) (Table 4).

Carrageenan induced rat paw edema was prevented (91.49% inhibition) by *P. javanica* leaf extract (400 mg/kg dose). This observation was comparable to the effect demonstrated by standard indomethacin (93.62% inhibition) (Table 5). A few anti-inflammatory compounds have been isolated from *Picrasma quassioides* (Jiao et al., 2011). *β*-sitosterol has been reported as a potent anti-inflammatory molecule (Nirmal et al., 2012; Gupta et al., 1980). In terms of modern medicines, drugs that exhibit analgesic activity, might also possess anti-pyretic and anti-inflammatory activities. Traditionally, *P. javanica* has been used in fever and malaria (Heyne, 1987; Zhang et al., 2018). The quassinoids and alkaloids which were found to be responsible for the anti-malarial potential, might be also responsible for the observed analgesic and anti-inflammatory effects (Saiin, 2017; Pavanand et al., 1988; Saiin et al., 2016). *P. javanica* leaf extract further enhanced the duration of phenobarbitone sodium-induced sleep (Table 6).

The outcomes of anti-diarrheal activity assay and

Table 3. Anti-nociceptive activity of methanolic crude extract of *P. javanica*.

Group	% Pain inhibition		
	30 min	60 min	90 min
Morphine (standard) (2 mg/kg)	72.52	139.06	70.33
Methanolic crude extract (200 mg/kg)	46.96	20.94	2.33
Methanolic crude extract (400 mg/kg)	49.68	33.13	17.0

Source: Authors 2022

Table 4. Effect of loperamide and *P. javanica* extract on castor oil (1 ml/mice) induced diarrhea in mice.

Group	Number of diarrheal faeces	% Reduction of diarrhea
Control (10 ml/kg)	16.83±0.08	-
Loperamide (Standard) (50 mg/kg)	4.81±0.12**	71.42
Methanolic crude extract (200 mg/kg)	6.78±0.03**	60.12
Methanolic crude extract (400 mg/kg)	6.24±0.40**	63.10

Values are expressed as mean ± SD; **p<0.01 vs. control.
Source: Authors 2022

Table 5. Effect of *P. javanica* extract on carrageenan induced paw edema in Wistar rats.

Group	Paw edema		% Inhibition of edema at 4th h after carrageenan injection
	At 1st h after carrageenan injection (cm)	At 4th h after carrageenan injection (cm)	
Control (100 ml/kg)	0.66±0.01	0.94±0.05	-
Indomethacin (Standard) (10 mg/kg)	0.44±0.20	0.06±0.03**	93.62
Methanolic crude extract (200 mg/kg)	0.62±0.03	0.17±0.14**	89.36
Methanolic crude extract (400 mg/kg)	0.56±0.12	0.08±0.03**	91.49

Values are expressed as mean ± SD; **p<0.01 vs. control.
Source: Authors 2022

Table 6. Effect of *P. javanica* leaf extract on phenobarbitone sodium-induced sleep.

Group	Time of onset of sleep (min)	Total sleeping time (min)
Control (10 ml/kg)	15.81±0.13	118.69±0.11
Crude methanol extract (200 mg/kg)	12.29±0.03	131.45±0.41*
Crude methanol extract (400 mg/kg)	10.06±0.23	150.63±0.20**

Values are expressed as mean ± SD; *p<0.05, **p<0.01.
Source: Authors 2022

phenobarbitone sodium-induced sleeping time test were new findings. It created the opportunity to search for the responsible phytocomponents.

Conclusion

The aim of the study was to determine the bioactivities of *P. javanica* leaf extract. This species exhibited very prominent analgesic, anti-inflammatory, and anti-diarrheal activities. Mankind has constantly been looking for remedies against different ailments and relied on natural sources for the treatment of diseases. Isolation and identification of the phytocomponents responsible for the observed activities in *P. javanica* leaf extract can be considered as promising arena for further investigation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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