Acta Manilana 68 (2020), 31–37

ISSN: 0065–1370

Acta Manilana

journal homepage: journals.ust.edu.ph

Antinociceptive effect of the ethanolic extract of *Pandanus panayensis* Merr.

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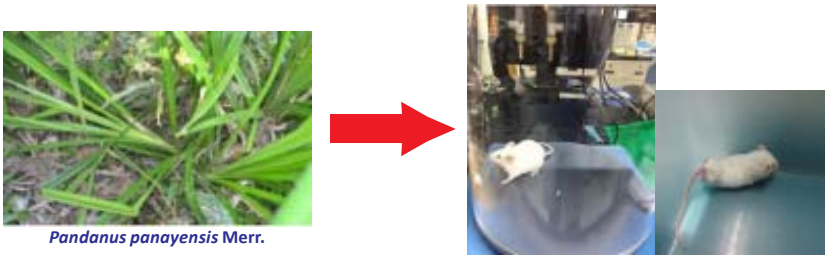
Graphical Abstract



Abstract

The current investigation explored the antinociceptive potential of the crude ethanolic (OHP) extract of *Pandanus panayensis* Merr. (Pandanaceae). Different doses (1000 mg/kg, 500 mg/kg, and 250 mg/kg BW) were used to establish their peripheral and central nociception activities using in vivo acetic acid-induced writhing and hot plate tests, respectively. Acetic acid-induced writhing test showed that the OHP extract at 500 mg/kg BW has the highest reduction in the number of writhes and is significantly comparable to the positive control. Hot plate test revealed very minimal or no central nociception for all doses. Hence, the OHP extract showed promising analgesic action through peripheral antinociception effects. Further pharmacological and chemical investigations are required to identify the bioactive metabolites and to characterize their mechanism of action.

Keywords: antinociception, *Pandanus*, *Pandanus panayensis*, crude extract

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Introduction

Pain is a natural body reaction from the activation of neurons to protect the body from any possible further damage [1, 2]. As pain affects different ages, it is one of the common symptoms, which people seek for medical attention due to possible underlying diseases. Although there are many available safely reported over-the-counter (OTC) painkillers like paracetamol, these agents can damage liver in chronic uses and unintended overdose [3]. To alleviate pain, anti-inflammatory drugs like NSAIDs are used, which also possess analgesic activity and selectively relieve pain without affecting consciousness [4, 5]. However, prolonged NSAIDS medication has been observed to show adverse effects like hypersensitivity, gastric ulceration and kidney problems. This led the current investigation to find promising medicinal plants to alleviate pain and associated health problems [5–7]. In connection, this scientific study was conducted to determine the analgesic activity of this endemic *Pandanus* species grown in the Philippines.

Genus *Pandanus* is composed of 700 species that can be found in tropical and subtropical regions. In the Philippines, there are 52 *Pandanus* species growing in various habitats. The word "pandans" that originated from Malay is a general name for all Pandanaceae family [8]. Scientific studies of different *Pandanus* species revealed antibacterial, anti-inflammatory, anti-diarrheal and cytotoxic properties, validating their traditional folkloric uses. Several secondary metabolites have been identified from *Pandanus* species such as steroids, terpenoids, flavonoids, lignans, benzenoids and alkaloids [9]. The identified compounds from traditional medicinal plants can be substantial in proving other pharmacological benefits [10]. *Pandanus panayensis* Merr. is one of the endemic species in the Philippines. Extensive literature survey also revealed a dearth on its scientific pharmacological activity. In our interest to explore *Pandanus* species for their anti-inflammatory potential, we herein describe the antinociceptive effects of the crude ethanolic extract of *P. panayensis*.

Materials and methods

Plant material and extraction. Fresh *P. panayensis* leaves were collected from Lauan, Antique. Identification was made by the botanist curator of the UST Herbarium where the voucher specimen was also kept (USTH014475). Air-dried and ground leaves of *P. panayensis* (2 kg) were extracted with 2.5 L of ethanol in a percolator overnight. The ethanolic fraction was filtered and collected. The process was repeated thrice to consume a total of 9 L ethanol. The combined ethanol fractions were evaporated under reduced pressure to remove all the ethanol solvent. The process yielded the crude ethanol *P. panayensis* (OHP) extract with a sticky and thick consistency and dark-green color. The OHP extract was kept in plastic bottles, weighed, labeled, and stored at –20°C for pharmacological testing.

Experimental animals. The protocol for the in vivo experiments for the antinociceptive activity was approved by UST Institutional Animal Care and Use Committee (IACUC) with Animal Research Permit No. AR-2018-355. Male ICR mice were acquired from the Research Institute for Tropical Medicine (RITM) in Alabang,

Muntinlupa, Philippines. All mice were aged five to six weeks weighing 27–38 g. The animals were divided in six mice per group and acclimatized at the UST-RCNAS Animal Holding Facility for not less than seven days prior to the experimentation. All were kept under standard environmental condition of temperature at 25°C and light/dark cycles (12/12 h) with free access to water and food.

Acetic acid induced writhing method. The acetic acid-induced writhing test is reactive at a dose level that may not respond in other testing method like hot plate [11]. This triggers the production of inflammatory mediators that induce pain sensation such as bradykinin and prostaglandins particularly PGI_2, PGE_2, and PGF_{2a} as well as cytokines like IL-β and TNF-α [11, 12]. The acetic acid induced writhing method was conducted based on a previous protocol [13] with modifications.

Healthy male ICR mice were grouped into five with six mice per group — (A) negative control group was given with 1% Tween 80 in normal saline (10 mL/Kg BW), (B) positive control group was treated with diclofenac sodium (40 mg/kg BW). The three groups were treated with different doses (C) 1000 mg/ Kg BW, (D) 500 mg/Kg BW, and (E) 250 mg/Kg BW) of OHP. One hour after oral administration of the test samples to the respective group, 0.6% acetic acid (10 mL/Kg BW) was injected intraperitoneally (i.p.). After 5 min, contraction of the abdominal muscles with the stretching of hind limbs was observed and counted in 20 min.

Hot plate test. The method used for the hot plate test was based on a previous study [4] with modifications. Pain reflex in response to the thermal stimulus was measured using an Ugo Basile Hot/Cold Plate 35100 (Comerio VA, Italy). Male ICR mice weighing 27–40 g were assigned randomly in five different groups, with five mice per group. Baseline latency of each mice was measured before induction. Each group was orally treated in the following manner: Group 1, negative control with 1% Tween 80 in saline solution (10 mL/kg BW); Group 2, positive control with morphine sulphate as reference drug (5 mg/kg BW); Group 3, OHP 250 mg/kg BW; Group 4, OHP 500 mg/kg BW; and, Group 5, 1000 mg/kg BW. Each mouse was placed on a 55 ± 1°C hot plate to obtain their response to electrical heat induced nociceptive pain stimulus. Nociceptive reaction was judged by the presence of behaviors such as licking of the fore and hind paws or jumping. The pain response was measured at 30 min and at every hour thereafter for 3 h. The cut-off time that was set to prevent skin damage was 30 sec.

Statistical analysis. Data were expressed as mean ± standard deviation of replicate analysis ($n = 6$ acetic acid writhing test, $n = 5$ for hot plate test). Single-factor analysis of variance (ANOVA), and Tukey's HSD were used to determine significant differences at $p < 0.05$ were analyzed using IBM SPSS Statistics version 21.

Results and discussion

The antinociceptive activity of the crude ethanolic (OHP) extract derived from *P. panayensis* was evaluated by using murine pain models, namely acetic acid- induced writhing and hot plate tests.

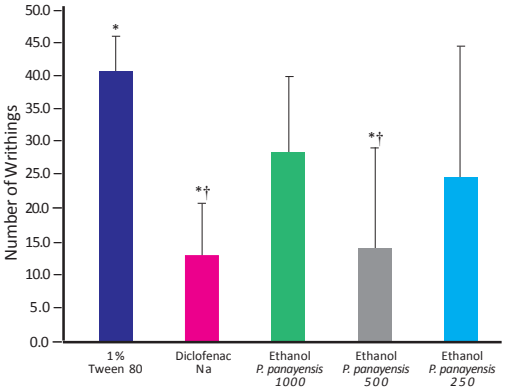

Figure 1. Acetic acid-induced writhes in 20 min post-sample administration. Results are expressed as mean ± SD of *n* = 6; p >0.05. (*Negative control is significantly different from diclofenac group with p = 0.010 and ethanol extract of *P. panayensis* 500 with p = 0.013; † ethanol extract of *P. panayensis* 500 is significantly similar with diclofenac p = 1.000)

Acetic acid writhing test. As shown in Fig. 1, results revealed that reduction of muscle constrictions induced by intraperitoneal administration of acetic acid was observed to be in a dose independent manner. The negative control was significantly different from the positive control with p-value of 0.010. Acetic acid induced writhing test of OHP 500 mg/kg BW showed a highly significant reduction in the number of writhes (14.17) and is statistically comparable to diclofenac sodium (p = 1.000). The positive control group, diclofenac at 40 mg/kg BW, showed the most reduced number of writhes at 13.33, which is a reduction of 67.62% compared with the negative group with 41.17 writhes. The OHP 1000 mg/kg BW showed the highest counts of writhes at 28.50 among the three doses and is statistically similar with the negative control (p = 0.475). A total of 25 writhes were counted for OHP 250 mg/kg BW, which is statistically comparable with the standard diclofenac sodium, but not as notable as OHP 500 mg/kg BW.

Hot plate test. Hot plate test, as shown in Table 1, revealed latency score exhibited by the positive control at 0.5 h is statistically higher than that of the negative control (p = 0.010). In the same time (0.5 h), the latency scores of the extracts are still statistically the same with that of the negative control (p = 0.592–1.000). The positive control, morphine sulfate, proved its immediate pain tolerance effect for the first 30 min. After 1 h and 2 h period, the latency score of the negative control is significantly lower than the latency score of the positive control (p = 0.000 and p = 0.015, respectively). This indicated that the pain tolerance caused by the drug is still efficient at this time. The groups treated with the different doses of the OHP resulted to minimal increase in latency scores and are statistically the same with that of the negative control (p = 0.167–0.895) at the first and second hour (p = 0.542–1.000). This showed insignificant increases in the latency scores when compared to the positive control (p < 0.05). The pain tolerance of the positive control group decreased on the third hour, however, the extract groups still showed low similarity in central antinociception.

Receptors that can detect noxious stimuli are called nociceptors. Mechanical pressure, temperature ranging from <10°C to >40°C in mammals, and even chemicals like acids can serve as sources of noxious stimuli, which can damage tissues [14]. The antinociceptive activity of the ethanol leaf extract of *P. panayensis* was assessed by acetic acid induced writhing and hot plate tests. Peripherally acting analgesics can be demonstrated by acetic acid induced writhing method where generation of pain occurs through endogenous mediators such as serotonins, bradykinins, substance P and prostaglandins [15–17]. This occurs when the body is injected with acetic acid,

Table 1. Latency score of each control group; Negative control (1% Tween 80 in NSS), Positive control (Morphine SO_4 5 mg/kg BW), ethanol extract of *P. panayensis* at 1000, 500, and 250 mg/ kg BW, from 0 up to the third hour.

Time (h)	Negative Control (1% Tween 80)	Positive Control (5 mg/Kg BW morphine sulfate)	250 mg/Kg Ethanol Extract *P. panayensis*	500 mg/Kg Ethanol Extract *P. panayensis*	1000 mg/Kg Ethanol Extract *P. panayensis*
0	6.70±1.69	8.62±0.98	9.72±2.10†	9.28±1.33	6.70±1.11
0.5	8.26±1.95*	16.02±5.45†	11.31±3.07	10.56±2.01	8.54±2.60*
1	7.96±1.58*	16.80±2.88†	10.96±1.97*	10.46±0.73*	9.10±2.23*
2	9.40±1.47*	16.12±3.78†	12.34±4.39	10.78±1.92	8.92±2.31*
3	9.26±2.01	12.82±3.14	11.58±1.78	11.10±1.85	7.76±1.64*

Results are expressed as mean +/– SD of $n = 5$ at $\alpha = 0.05$. The (*) indicates statistically comparable results with the negative control and (†) indicates statistically comparable to the positive control at the same row, $p < 0.05$.

arachidonic acid is released causing production of prostaglandins from the phospholipids as mediated by enzymes, phospholipase A_2 and acyl hydrolases. Prostaglandins, thromboxane and prostacyclin are generated through cyclooxygenase pathway [17–19]. Prostaglandins PGI_2 and PGE are responsible for stimulating pain in the nerve endings of the visceral organs [18]. This in vivo test illustrated that OHP can reduce the number of writhings, which may indicate that the analgesic activity of the OHP extract is by peripheral mechanism. Compared with the positive control, diclofenac sodium (40 mg/kg BW), the 500 mg/kg BW extract dose showed the highest reduction in the number of writhes and is considered to be the optimum dose, which can exhibit peripheral antinociception [13]. This also indicates that inhibiting the production of prostaglandins is a significant antinociceptive mechanism for visceral pain [17].

Hot plate test is a standard method to evaluate central analgesic activity as shown by opioids. Opioid receptors react selectively with narcotic analgesics [11, 16, & 20]. Opioid receptors respond to three major receptors, m, d, and k receptors and their ligands b-endorphin, enkephalin and dynorphin. These ligands are mostly distributed in central and peripheral nervous system [21]. Morphine responds to μ receptor [14]. *Pandanus panayensis* crude ethanol extract apparently did not prolong the latency time and acted similarly with negative control, indicating minimal or negative central nociception. Hence, results may suggest that the analgesic action of *P. panayensis* crude ethanolic extract is limited by peripheral antinociception as shown by its significant reduction in the number of writhes.

Several *Pandanus* species have also shown to exhibit antinociceptive activity. Chloroform extract of *P. fascicularis* was found to have significant antinociceptive effect at the dose levels of 100, 200, and 400 mg/kg, orally in mice [22]. Methanol extract of *P. foetidus* produced a significant step-down in pain using the formalin test, while the chloroform fraction maximally reduced the heat-induced analgesia in the tail immersion test [23]. Ethanolic extract of *P. tectorius* showed analgesic activity at 300 mg/kg and is comparable to ibuprofen [24, 25]. Hence, *P. panayensis* adds to the list of biologically-active *Pandanus* species with promising antinociceptive property.

Conclusion

Taken together, *P. panayensis* extract exhibited an analgesic property by inhibiting peripheral nociception. This is the first report on the biological activity of *P. panayensis* and adds to the number of *Pandanus* species exhibiting analgesic property. Pharmacological and chemical investigations are required to identify the bioactive metabolites and to characterize their mechanism of action.

Acknowledgments

The Commission on Higher Education (CHED) Dare-2 grant is acknowledged for the financial support. The Thomasian Angiosperm Phylogeny and Barcoding Group, headed by Prof. Dr. Grecebio Jonathan Alejandro, is also gratefully acknowledged for the collection of plant material.

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