International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1146-1151 www.ijprajournal.com ISSN: 2456-4494

Phytochemical screening and analgesic activity of ethanol extract of Vitex trifolia

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Submitted: 01-12-2022 Accepted: 10-12-2022

ABSTRACT:
The pain was considered an inevitable sensory response to tissue damage. Pain is probably the most common symptomatic reason to seek medical consultation.However, conventional painkillers are ineffective in treating all types of pain, additional efforts have been made to develop analgesic drugs from natural materials. In this study, the ethanolic extract of Vitex trifolia (EEVt) was examined for its analgesic activity at doses of 150, 300, and 600 mg/kg body weight. Acetic acid-induced writhing was used to evaluate the analgesic activity of EEVt. The results showed that the EEVt(150, 300, and 600 mg/kg BW) had significant analgesic activity. The percentage of inhibition was 19%, 36%, and 49%, respectively. These findings imply that Vitex trifolia leaves extracts have promising analgesic properties.

KEYWORDS: Antinociceptive, Analgesic, Vitex trifolia, Legundi

I. INTRODUCTION

Pain is an unpleasant sensation that is usually brought on by intense or harmful stimuli. It is also defined as a distressing sensory or emotional experience linked to actual or potential tissue damage.[1]. Pain is known as a complex experience with motivational, emotional, sensory-discriminative, affective, and cognitive components[2], [3]. Pain is sometimes the only symptom used to diagnose multiple diseases.[4], [5]. Throughout history, Humans have used many forms of therapy for pain relief, with medicinal plants being one of the most common and widely used.[6]–[9]. The exploration of natural compounds with comparable analgesic activity but fewer side effects is important.

For thousands of years, medicinal plants have been used to treat a variety of human ailments, and they provide a rich source of novel therapeutics.[7], [9]–[12]The genus Vitex (Verbenaceae) includes approximately 250 species distributed in the tropical and subtropical regions of the world[13]. Vitex trifolia is often found in Southeast Asia, Micronesia, Australia, and East Africa, and its fruits have been commonly used as a folk medicine for the treatment of headaches, colds, migraine, and eye pain in China.[14].

The Vitex trifolia has been extensively investigated to result in the isolation of flavonoids [15], diterpenes[16], and alkaloids[17], and some of these constituents exhibited significant pharmacological activities, such as anti-inflammatory[18], antilarvicidal[19] anti-oxidative [20], and antitumor [21] effects. In the current research, we have now evaluated the phytochemical screening and the potential analgesic activity of the ethanolic extract of Vitex trifolialeaf on acetic acid-induced writhing models.

II. MATERIALS AND METHOD

Materials

The following drugs and chemicals were used in the current study: ethanol 95% (PT. Novalindo), Aspirin (acetylsalicylic acid) (PT. Darya-Varia Laboratoria Tbk), Acetic acid (Merck, Germany) and other reagents were purchased from Bratachem (Indonesia).

The Vitex trifolialeaveswere collected from Rokan Hulu, Riau, Indonesia. The Vitex trifolia was identified by Dr. Nurainas, a botanist at the Herbarium of Andalas University, West Sumatera, Indonesia.

Preparation of The Ethanol Extract of Vitex trifolia(EEVt)

The Vitex Trifolialeaves were sun-dried. The dried Vitex trifoliawas powdered using a conventional grinder. The powdered materials were then soaked in ethanol (95%) for 24 hours by stirring at room temperature. The materials were filtered after 24 hours. The procedure was repeated three times. The filtrates were mixed and concentrated under a vacuum using a rotary until free of solvent. The extract was kept cold for further pharmacological testing.

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Phytochemical screening

EEVt was qualitatively tested for the detection of saponins, flavonoids, Phenolic, tannins, alkaloids, phenolic, terpenoids, and steroids following standard procedures [22].

Experimental Animal

15 adult male mice with body weights of 20–25 g and aged 2-3 months were obtained from West Sumatera animal houses and were used for this study. Animals were housed and cared for in standard conditions with 12 h light/dark circle and were fed with a standard pellet diet and water ad libitum. All the animals were acclimatized for a minimum period of 1 week prior to the experiment. After 1 week, animals were randomly selected for different experimental groups (3 animal/ group) and used for the in vivo determination of antinociceptive activity. The rats were deprived of food, but not water, for 18–20 hours before an experiment. The protocol of this experiment was approved by The Committee of The Research Ethics of The Faculty of Medicines, Andalas University (permit No. 336/KEP/FK/2020).

Analgesic activity:

Acetic acid-induced writhing model was used for evaluating the potential of ethanolic extract of the plant on pain. In this method, pain was produced by the administration of 1% v/v of acetic acid (1mL/100g body weight of mice). The mice were placed in separate boxs under observation immediately after the acetic acid injection and a number of abdominal constrictions were counted over a period of 30 min. The experimental protocol comprises as follows:
Group I (Control, Na.CMC 0,5%)
Group II was treated with EEVt (150mg/kgBW, orally)
Group III was treated with EEVt(300mg/kgBW, orally)
Group IV was treated with EEVt(600mg/kgBW, orally)
Group IV was treated with Aspirin (65mg/kgBW, orally).
The groups used for observing the influence of ethanolic extract on 1% v/v of acetic acid-induced writhing in mice. Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted.

The percentage protection was calculated by following the formula:

$$\% \text{ Analgesic Activity} = \frac{\text{Mean writhing count (control} - \text{Treated)}}{\text{Mean writhing count control}} \text{ x } 100$$

Statistical Analysis

The statistical software SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Data were analyzed using one-way ANOVA followed by Duncan's multiple range test. In all tests, the criterion for statistical significance was $p < 0.05$.

III. RESULTS

Phytochemical screening

In the current study, preliminary phytochemical screening tests of the crude extract showed the presence of alkaloids, flavonoids, saponins, terpenoids, phenolic and tannins. (Table 1).

Table 1. Phytochemistry screening test result of Vitex trifolia

Groups	Result
Alkaloid	+
Falvonoid	+
Saponin	+
Steroid	-
Terponoid	+
Phenolic	+
Tannin	+

Analgesic activity

Vitex trifolialeaves presented significant analgesic activity to the control group in test models of nociception induced by chemical agents. In the acetic acid-induced writhing test, performed in the present study, EEVt in the doses of 150, 300 and 600 mg/kg, p.o., significantly reduced the number of writhes (24,4 ± 5,7;19,2 ± 1,8; and 15,4 ± 2,8writhes/30 min), respectively, in relation to the control group (30 ± 1,5writhes/30 min) (Fig. 1). The Aspirin (65 mg/kg, p.o.), a nonsteroidal anti-inflammatory drug, also promoted a significant

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reduction in the number of writhes (13,1 ± 1,7writhes/30 min). The percentage inhibition of pain was calculated as 56,33% (Aspirin), 19% (EEVt6150 mg/kg), 36% (EEVt300 mg/kg), and 49% (EEVt600 mg/kg) (Table 2).

Tabel 2. Analgesic Activity by Acetic Acid Induced Writhing in Mice of Vitex trifolia

Gruops	Treatment	Dose (mg/kg B.W)	Writhings	(%)inhibition[a]
I	Control (Na. CMC 0,5% + Acetic Acid 1%)	-	30 ± 1,5	-
II	EEVt	150	24,4 ± 5,7	19*
III	EEVt	300	19,2 ± 1,8	36*
IV	EEVt	600	15,4 ± 2,8	49*
V	Aspirin[b]	65	13,1 ± 1,7	56*

[a]Data are expressed as the mean of Three observations (n = 3), [b]Used as comparative group
*** Significant difference compared to the positive control (P < 0.05)**

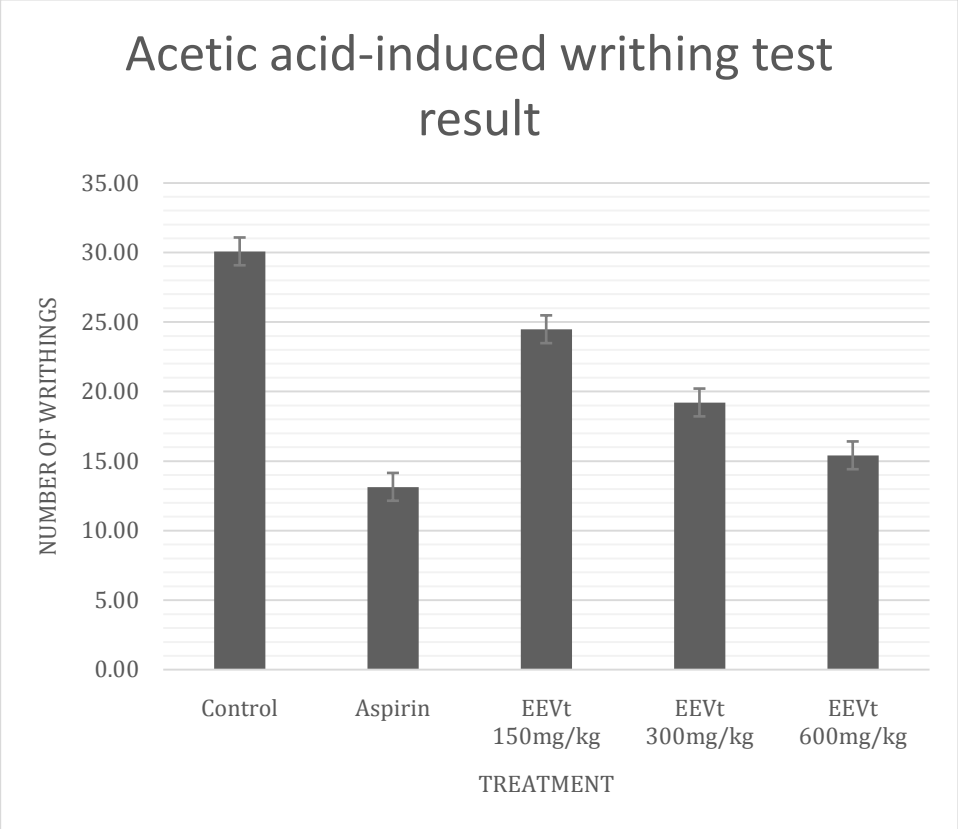

Figure 1. The antinociceptive effect of ethanol extracts Vitex trifoliaon the acetic acid-induced writhing test.

IV. DISCUSSION

The current research showed that administering EEVt orally has a potent and dose-dependent analgesic effect in chemical-induced nociception models. In the acetic acid-induced writhing test, performed in the present study, EEVtin the doses of 150, 300 and 600 mg/kg, p.o., significantly reduced the number of writhes.

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In chemical nociception, the writhing action in mice caused by an intraperitoneal injection of acetic acid is used to assess central and peripheral analgesic activity.

Acetic acid administered intraperitoneally raises the levels of cyclooxygenase (COX), lipoxygenase (LOX), prostaglandins (PGs), histamine, serotonin, bradykinin, substance P, IL-1 beta, IL-8, and TNF alpha in peripheral tissue fluid[23].Increased level of these mediators causes the excitation of primary afferent nociceptors entering dorsal horn of the central nervous system[24].

In accordance with the percentage of inhibition of the number of the writhes obtained through Vitex trifolia use, it was observed that the intensity of its analgesic effect was almost close to the Aspirin. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) can inhibit cyclooxygenase (COX) in peripheral tissues, reducing the synthesis and/or release of inflammatory mediators and thus interfering with the mechanisms of primary afferent nociceptors' transduction.[25]The analgesic mechanism of action of the Vitex trifoliacan, probably, involve inhibition of the synthesis and/or release of inflammatory mediators who promote pain in the nervous terminations, similarly to the Aspirin and the other NSAIDs suggesting a peripheral analgesic action. However, the test of abdominal constrictions is non-specific, since NSAIDs and opioid analgesics may inhibit the nociceptive response in the acetic acid model[26], [27].

According to our phytochemical screening results, EEVtcontains terpenoidOther studies suggested that plant materials that contain triterpenoidpossess analgesic and anti-inflammatory effects on experimental animals and these pharmacological effects are resulted from these contents[16], [28]–[30]. Additionally, different terpenoids have been found to be antinociceptive and anti-inflammatory agents due to their ability to inhibit arachidonic acid metabolism[31]–[35]. Therefore, it is possible that the presence of terpennoids in the EEVtmay be responsible for the antinociceptive effect.

V. CONCLUSION

Vitex trifolia leaves ethanol extract had a significant and dose-dependent analgesic effect. The 600 mg dose has shown better potency. More research is required to confirm this preliminary finding, which could support some of the plant's uses in Indonesian herbal medicine practice.

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