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Analgesic Activity of Infusion of Beluntas Radix (*Pluchea indica* (L.)) on the Male Mice

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***Abstract*—Objectives: The study aims to determine the analgesic activity of beluntas radix infusion. Method: The research used 25 of male mice. The mice were divided into five groups, which are negative control, positive control, and three testing groups (in different doses). Fifteen minutes after infusion of beluntas radix per oral, the mice were given the acetic acid 0.7% i.p. The cumulative writhe was calculated in every 5 minutes for one hour and was counted for the protection and the effectivity percentage. Results and Discussions: Beluntas radix infusion of doses 9.75 mg/20 g, 19.5 mg/20 g and 29.25 mg/20 g of body weight decreased the writhing responses significantly ($p<0.05$) compared with the control. Each doses gave the protection percentage 55.8%, 64.2% and 77.5% and the effectivity percentage 113.4%, 130.5% and 157.5%. Conclusion: Analgesic activity of beluntas radix infusion is influenced by dose level. Test dose III (29.25 mg / 20 g BW of mice) has the best activity.**

Keywords: radix,* Pluchea indica L.*, infusion, analgesic, writhing method

I. INTRODUCTION

Natural resources were known as a traditional medicine which used traditionally based on personal experience or the other experiences [1]. One of them is beluntas (Pluchea indica L.) which contain many chemical compounds such as flavonoid and tannin. Beluntas are wildly found in nature or in the environment around humans. Beluntas has some efficacy such as antibacterial, deodorant, halitosis, appetite supplement, digestive diseases of children, tuberculosis, rheumatism, bone, and low back pain [2].

Beluntas roots are used to treat the pain by the people [2]. Scientific proof of beluntas root activity against pain reduction needs to be evaluated, so this study aims to make sure clearly its efficacy as an analgesic.

II. MATERIAL AND METHOD

A. Plant Material

Beluntas roots from Tasikmalaya city are used as the plant material and then it's determinated in the Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor.

B. Animals

A total of 25 Swiss Webster strains of male mice weighing between 20 and 30 g were used as the experimental animals. The mice were purchased from School of Life Science, Institute Teknologi Bandung, Indonesia. The mice have housed in wire mesh cages under standard conditions (temperature, 25-30°C, 12 hrs lights, and 12 hrs dark cycles) and allowed to acclimatize for five days. The mice were fed with standard pellets diet, and water was given *ad libitum*.

C. Chemicals

Acetic acid, Paracetamol, Pulvis Gummi Arabicum (PGA), water, aquadest, Chloroform, HCl, ammonia, Dragendorff reagent, Mayer reagent, Bouchardat reagent, Lieberman-Burchard reagent, Mg metal, $FeCl_3$, gelatin, amyl alcohol, ether, NaOH, H_2SO_4, vanillin, anhydrous acetic acid.

D. Infusion Preparation

Beluntas root collection, wet sorting, washing, chopping, making 10% infusion w/v from Simplicia powder.

E. Phytochemical Screening

Phytochemical screening aims to determine the class of chemical compounds of beluntas radix such as alkaloids, flavonoids, tannins, saponins, steroids/terpenoids, quinones, and monoterpene/sesquiterpenes.

F. Analgesic Activity Test

The infusion was given to mice with a dose I of 9.75 mg/20 g body weight of mice, dose II of 19.5 mg/20 g body weight of mice and dose III of 29.25 mg/20 g body weight of mice.

Mice were randomly divided into five groups; each group contained five mice. The negative control group was only given 2% PGA solution, the positive control group was given paracetamol suspension and the tests group which were given infusion suspension for dose I of 9.75 mg/20 g body weight of

mice, dose II of 19.5 mg/20 g body weight of mice and dose III of 29.25 mg/20 g body weight of mice.

All treatments were given orally as much as 0.3 ml. After 30 minutes, the mice were induced with 0.7% acetic acid as much as 1 ml/20 g BW of mice intraperitoneally. The amount of writhe was observed and counted for 60 minutes, with an interval of five minutes.

III. RESULTS

Phytochemical screening is carried out on several classes of secondary metabolites. The results are shown in Table I, and the compounds with positive results are predicted as the analgesic activity contributor.

TABLE I . PHYTOCHEMICAL SCREENING OF *P. INDICA* L. RADIX NFUSION

Secondary Metabolite	Results
Alkaloid	-
Saponin	+
Seskuiterpene/monoterpene	-
Tanin	-
Polifenol	+
Steroid/triterpenoid	-
Quinon	+
Flavonoid	+

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The Analgesic activity was shown from the test sample ability to reduce the amount of writhing after pain induction each interval time. The data were analyzed with One Way ANOVA and post hoc LSD. The test results are shown in Table II.

TABLE II. RESULTS OF WRITHING AMOUNT TEST

Group	Average of Writhing amount ± SD
Positive (PCT 1.3 mg/20 g BW of mice)	8.03 ± 0.73*
Negative (PGA 2 %)	15.8 ± 1.25
Test I (infusion of *P. indica* radix 9.75 mg/20g BW of mice)	6.98 ± 1.03*
Test II (infusion of *P. indica* radix 19.5 mg/20g BW of mice)	5.65 ± 0.79*
Test III (infusion of *P. indica* radix 29.25 mg/20g BW of Mice)	3.55 ± 1.54*

* Results are expressed as mean±SD *explain significant difference compared with the negative control

Positive, test I, test II, and test III group provides a decrease in writhing amount was significantly ($p<0.05$) when compared to the negative control group. It means that the administration of paracetamol and P. indica infusion can reduce the pain.

The protection percentage was obtained by comparing the writhe amount of the test group to the negative control group. The results are shown in Table III.

TABLE III. THE PERCENTAGE OF PAIN PROTECTION

Group	Pain Protection (%)
Positive (PCT 1.3 mg/20 g BW of mice)	49.2
Negative (PGA 2 %)	-
Test I (infusion of *P. indica* radix 9.75 mg/20g BW of mice)	55.8
Test II (infusion of *P. indica* radix 19.5 mg/20g BW of mice)	64.2
Test III (infusion of *P. indica* radix 29.25 mg/20g BW of Mice)	77.5

All of the test group gave a better percentage of pain protection than paracetamol. The percentage of pain protection was influenced by the test dose.

The analgesic effectivity was obtained by comparing the writhe amount of the test group to the positive control group. The results are shown in Table IV.

TABEL IV. THE PERCENTAGE OF ANALGESIC EFFECTIVITY

Group	Analgesic Effectivity (%)
Test I (infusion of *P. indica* radix 9.75 mg/20g BW of mice)	113.4
Test II (infusion of *P. indica* radix 19.5 mg/20g BW of mice)	130.5
Test III (infusion of *P. indica* radix 29.25 mg/20g BW of Mice)	157.5

Test III group had the highest percentage of analgesic effectivity. This effectivity was influenced by the test dose.

IV. DISCUSSION

This test used the Sigmund method (stretching method) because of its sensitivity to assess pain induction. Acetic acid as a pain inductor is good enough to trigger a local inflammatory response due to the release of free arachidonic acid from phospholipid tissue through cyclooxygenase isoenzyme (COX) and prostaglandin biosynthesis. Prostaglandins cause the pain and inflammation by increase the permeability of the peritoneal cavity. Pain response is indicated by stretching the leg and attaching the stomach to the floor [3].

The test results showed that the test dose affected the amount of mouse writhe. The higher the dose given, the smaller the amount of writhe. The smaller the amount of writhe, the higher the analgesic activity. The concentration of active compounds of each dose is predicted as a causative factor to the analgesic activity difference. Moreover, the nonoptimal administration time of acetic acid can affect the results because the onset doesn't reach yet.

Analgesic activity of dose II and III tests are better than positive controls because statistically, the LSD test showed a significant difference in the writhe amount ($P <0.05$). It means

that the beluntas radix infusion could be an alternative choice for natural analgesic drugs.

The chemical compounds of radix infusion are various, but the main compound that is predicted as a contributor of analgesic activity is flavonoids. Flavonoids are one of the phenolic compounds which have the most important effect as an antioxidants [4]. Flavonoids as antioxidants are anti-inflammatory agents that work through the capture of oxygen radicals released by peroxide. The pain due to cell membranes damage is caused by oxygen radicals [5];[6]. Moreover, flavonoids show anti-inflammatory, antiallergic, antiviral, and anticarcinogenic effects.

The anti-inflammatory effect of flavonoids originates from the inhibition of the enzyme system in metabolizing arachidonic acid. The release of Arachidonic acid is a starting point for the general inflammatory response. This indicates that flavonoids are anti-inflammatory Thus if the release of arachidonic acid is inhibited, the formation of prostaglandins (as a pain mediator) does not occur so the pain receptors stimulation of by prostaglandins can be inhibited.

V. CONCLUSION

Analgesic activity of beluntas radix infusion is influenced by dose level. Test dose III (29.25 mg / 20 g BW of mice) has the best activity.

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