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Evaluation of Analgesic Effect of Corn Silk Infusion (*Zea mays* L.)

Nidaan Khafiya[1], Andri Tilaqza[2]*, Reza Hakim[1]

[1]Medical Departement, Faculty of Medicine, University of Islam Malang, Malang 65144, Indonesia
[2]Pharmacy Department, Faculty of Medicine, University of Islam Malang, Malang 65144, Indonesia

Corresponding author:
E-mail:
andri.tilaqza@unisma.ac.id

ABSTRACT

Corn silk (*Zea mays* L.) is a plant that is widely found in Indonesia but is still unexplored. In silico studies show that corn silk contains compounds that have the potential as analgesics by inhibiting the work of the enzyme cyclooxygenase (COX) which can reduce the production of prostaglandins by arachidonic acid, but these studies have not been proven in vivo. This study aims to evaluate the analgesic effect of corn silk infusion. Corn silk was extracted by the infundation method. A phytochemical screening test was conducted to determine the content of corn silk infusion. Analgesic effect was evaluated using analgesimeter rendal-selitto in 30 male wistar rats. The negative control group was given distilled water, the treatment groups were given 125, 250, and 500 mg/kgBW of corn silk infusion, and the positive control group was given mefenamic acid suspension of 45 mg/kgBW. Corn silk infusion contains flavonoid, saponin, and alkaloid compounds based on phytochemical screening tests. The administration of corn silk infusion with doses of 125, 250, and 500 mg/kgBW produced a percentage of pain inhibition at 13.2%, 13.89%, and 20.42% compared to mefenamic acid at 25.11%. The greater analgesic activity was observed by the maximum dose of the infusion (500 mg/kgBW). The effect of the infusion was also statistically significant ($p<0.05$) only in the maximum dose. The result obtained from this study shows that the Corn silk infusion contained phytochemical constituents with analgesic activities, therefore could be used in the management of pain conditions. Further research is needed regarding the toxicity test and its mechanism of action.

Keywords: Analgesic, infusion, Zea mays L, corn silk

Introduction

Pain is a form of sensory and emotional discomfort that arises due to subjective tissue damage (Hall et al., 2013; IASP, 1979). Pain can be acute which has a short period or chronic pain which lasts more than three to six months (Dureja et al., 2017; Straube et al., 20211). In the inflammatory process, the pain process occurs due to nociceptor stimulus due to the release of various inflammatory mediators in the cyclooxygenase (COX) pathway in arachidonic acid metabolism (Kapur et al., 2021; Kumar et al., 2013).

Analgesics are a type of drug that is useful for reducing pain (Mancano & Gallaghe, 2019). Suboptimal pain management can prolong healing time which can lead to chronic pain (Aisyah, 2017). The side effects caused by analgesics are very detrimental if used excessively and prolonged, such as gastric irritation, impaired platelet function, urticaria, dry mouth, bronchial asthma, hypotension to shock, and even addiction (Ali et al., 2016). Thus, alternative analgesic drugs that have the smallest side effects are needed.

Corn silk (*Zea mays* L.) have been shown to have potential as an analgesic (Okokon et al., 2016). Compounds in ethanol extracts of corn silk (*Zea mays* L.) that have an analgesic role are flavonoids, alkaloids, and saponins (Okokon et al., 2016). These compounds are also found in ethanol, methanol, and decocta extracts of corn silk (*Zea mays* L.) (Singh et al., 2022; Sholihah et al., 2022). Flavonoid, alkaloid, and saponin compounds are known to play a role in inhibiting the work of the cyclooxygenase (COX) enzyme which can reduce arachidonic acid in producing

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prostaglandins so that pain is reduced (Octavianus & Lolo, 2014; Tamimi et al., 2020; Wemay et al., 2013). However, no research proves that these compounds are found in corn silk infusion (*Zea mays* L.) and have potential as analgesics.

Based on the principle of like dissolve like, a solvent tends to dissolve compounds that have the same level of polarity as the solvent (Khafidhoh et al., 2015; Simorangkir et al., 2019). Flavonoid compounds, alkaloids, and saponins are polar (Bintoro et al., 2017; Hammado & Iling, 2013; Koirewoa et al., 2012; Lestari & Sidik, 2013). One polar solvent is water (Sulistyarini et al., 2020). Infusion is an extraction method that requires water by heating to a temperature of 90 ℃ for 15 minutes (Irianto et al., 2020). The infusion method has advantages including using simple tools, easy, inexpensive to use, and closer to the traditional way of making medicines, namely boiling, although boiling is not recommended because it can reach a temperature of 100 ℃ which can damage the content of the compounds in it (Owoyele et al., 2010).

The paw pressure test is one of the analgesic test methods that has the advantage of being commonly used, simple, and effective so that it is easy to use, does not need to be calibrated before use, and is suitable for white rats (Santos-Nogueira et al., 2012). There has been no research using corn silk infusion (*Zea mays* L.) on male white Wistar rats with the paw pressure test method using a sliding load analgesy meter as an analgesic, so that is the background of this research.

Material and Methods

Research design

In vivo, laboratory quasi-experimental research was used in this study to test the potential of corn silk infusion (*Zea mays* L.) that can be utilized as an alternative analgesic in Wistar male white rats with the Paw Pressure Test method using a sliding load analgesy meter. Ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Islamic University of Malang with number 049/LE.001/X/03/2022 has been obtained for this study.

Research time

The research process was carried out in February and May 2023. Processing of corn silk (*Zea mays* L.) into simplisia as well as determination was carried out by the Batu Materia Medika Center, East Java. Furthermore, infusion making, phytochemical screening testing, and analgesic testing were carried out at the Integrated Laboratory of the Faculty of Medicine, Islamic University of Malang and the Biomedical Laboratory of the Faculty of Medicine, Islamic University of Malang.

Corn silk infusion (Zea mays L.) processing

Simplisia of corn silk (*Zea mays* L.) was obtained from the Batu Materia Medica Center with certificate number 074/653/102.20-A/2022. Simplisia of corn silk (*Zea mays* L.) with infundation method, using a ratio of 1:20 for the water solvent (Purnomo & Tilaqza, 2022). Simplisia that has been weighed is put into an infusion pot, then added distilled water. Then it was heated on the stove for 15 minutes, the calculation of which began when the temperature reached 90°C while stirring several times. After 15 minutes, the infusion pot was removed from the stove and the infusion was filtered using flannel (Lara & Elisma, 2021). The results of corn silk infusion (*Zea mays* L.) that have been obtained are then put into a freeze dryer for several days until a flat dry extract is formed thoroughly (Lestari et al, 2013; Oktavia et al., 2020). This is done so that the compounds contained therein are stable because they will be stored for some time. The resulting dry extract has a fainter color and a smoother texture compared to the extract before freeze-drying. Furthermore, it is stored in a tightly closed container.

Phytochemical screening of corn silk infusion (Zea mays L.)

Phytochemical screening was conducted to test the content of alkaloids, phenols, saponins, steroids, terpenoids, and flavonoids (Oktavia et al., 2020; Harborne, 1987). 10 ml of infusion was prepared, as well as a mixture of chloroform and water solvents in a ratio of 40 ml: 40 ml. Then

put everything gradually into a separating funnel. Next, shake gently. Then, the separatory funnel is waited until 2 layers appear, namely the chloroform layer and the water layer. Both layers were transferred into different measuring cups. The water phase is used to test flavonoids, phenols, and saponins. While the chloroform phase is used to test alkaloids, terpenoids, and steroids.

a. Flavonoid Testing
   4 ml of water phase was taken, then poured into a test tube. Then 1 ml of 0.05N NH_3 solution was added, then shaken. Flavonoid positive results when a yellow color appears.
b. Phenol Testing
   2 ml water phase was taken and poured into a test tube. Next, $FeCl_3$ 10% as much as 2 drops is added. Positive phenol causes a blue or purplish-blue color.
c. Saponin Testing
   2 ml water phase is taken and poured into a test tube. Then, shaken vigorously. The formation of foam can last for 15 minutes and when added 1 drop of concentrated HCl does not disappear, then the test results are positive for saponins.
d. Alkaloid Testing
   2 ml of chloroform phase was taken, then 1 ml of NH_3 0.05N and 1 ml of H_2SO_4 2N were added. Furthermore, 2 drops of Mayer reagent were given. The presence of a white precipitate indicates positive alkaloids.
   The chloroform phase was taken as much as 2 ml, then added 1 ml NH_3 0.05N and 1 ml H_2SO_4 2N. Furthermore, 2 drops of Dragendorff reagent were given. The presence of an orange-red-brown precipitate indicates positive alkaloids.
e. Steroid and Terpenoid Testing (Liebermann Burchard Test)
   A total of 3 drops of chloroform phase were placed in 2 holes of the drip plate. Then 2-3 drops of 1N acetic acid were added in one hole, while in the other hole 1-2 drops of concentrated H_2SO_4 were added as a comparison. The part of the hole that was given 1N acetic acid was stirred gently until dry. Next, 1-2 drops of concentrated H_2SO_4 were added. Then observations were made on the color formed. Red or purplish-red color gives positive results for terpenoids while steroids will be green or bluish green.
f. Terpenoid Testing (Salkowski Test)
   A total of 5 ml of chloroform phase is poured into a test tube. Then 3 ml of concentrated H_2SO_4 is added so that 2 layers of liquid phase will form. Between the layers, a reddish-brown color is formed which gives a positive result for terpenoids.
   Mefenamic acid was prepared in suspension form with CMC-Na according to the effective dose given to humans, which is 500 mg. The conversion factor used is 0.018, where the dose of humans with a body weight of 70 kg is converted to rats weighing 200 grams. With a conversion factor of 0.018, the dose used was 45 mg/kgBW of rats.

Dose determination and preparation of mefenamic acid suspension

The mefenamic acid suspension was made by weighing 500 mg of mefenamic acid and CMC-Na. Furthermore, the measuring cup is calibrated on the beaker glass and marked to give limits according to the calculated dose. Hot water was prepared and poured into the mortar. Then, CMC-Na was sprinkled on top and stirred until homogeneous. Waited for 10-15 minutes until the color was clear and expanded to resemble a gel. Next, mefenamic acid tablets that have been mashed are included, and then a little hot water is added, then stirred. The suspension was poured into a beaker glass. Hot water is added little by little until the mark limit has been made while still stirring.

***Dose determination of corn silk infusion* (*Zea mays* L.)**

The dose of corn silk infusion (*Zea mays* L.) used is 125 mg/kgBW, 250 mg/kgBW, and 500 mg/kgBW. For the manufacture of infusion weighed based on the calculation of the dose and adjusted to the body weight of each rat. The basis for selecting the dose of corn silk (*Zea mays* L.)

is the dose of corn leaves (*Zea mays* L.) which has potential as an analgesic (Okokon et al., 2016). Calculation of the dose of corn silk infusion (*Zea mays* L.) is done by calculating the preparation used first, then calculating the volume to be injected based on the body weight of each rat.

Animal preparation

Wistar male white rats with a body weight of 150-200 grams in a healthy condition (fur does not fall out, not dull, has clear eyes) were used in this study (Owoyele et a;., 2010; Asmara et al., 2017). Rats were measured for body weight then separated into five different cages and labeled to facilitate grouping. Each group contained 6 rats. For ± 18 hours before testing, the rats were fed while still given a drink (Rochma et al., 2022). Labeling of rat groups as in the information below..

PC = positive control group given mefenamic acid suspension 45 mg/kgBW
NC = negative control group was given distilled water
P1 = dose group of corn silk infusion (*Zea mays* L.) 125 mg/kgBW
P2 = dose group of corn silk infusion (*Zea mays* L.) 250 mg/kgBW
P3 = dose group of corn silk infusion (*Zea mays* L.) 500 mg/kgBW

Analgesic *testing*

Corn silk infusion (*Zea mays* L.) consisting of three doses (125, 250, and 500 mg/kgBW), mefenamic acid suspension 45 mg/kgBW rats, and distilled water were given orally using a sonde before testing. The principle of analgesic activity testing is clamping on one of the soles of the foot in between or the membrane of the rat's toes with a load (gram) using a sliding load analgesy meter at 30, 60, 90, 120, 150, 180, 210, and 240 minutes. The load was attached and then pressed when the membrane of the rat's toe was placed on the clamp. The load will continue to move according to the scale shown. Normally on a scale ≥6 with 1 weight (10 grams) the rat has a paw withdrawal response, so the load will stop and the resulting pain threshold can be recorded (Santos-Nogueira et al., 2012). Pain threshold is the amount of load that can be withstood by the rat when it pulls its paw. The pain threshold is expressed in Area Under the Curve (AUC) which can then be calculated as a percentage of pain inhibition.

$$\mathbf{AUC}_{n-1}^{n} = \frac{\mathbf{X_{n-1}+X_n}}{\mathbf{2}} \ (\mathbf{t_n - t_{n-1}})$$

Note:

AUC pain threshold	=	*area under curve* (gram. minutes)
X_n	=	pain threshold 30 minutes after time
X_{n-1}	=	pain threshold 30 minutes before time
t_n	=	after 30 minutes
t_{n-1}	=	before 30 minutes

$$\textbf{Percentage of pain inhibition} = \frac{\mathbf{B-A}}{\mathbf{B}} \ \boldsymbol{x}\ \mathbf{100}$$

Note:

A = The AUC pain threshold of the negative control group
B = The AUC pain threshold of the treatment group

Data analysis

One Way ANOVA test was conducted on the Area Under Curve (AUC) value of pain threshold because the data distribution was found to be normal. Furthermore, the Least Significance Different (LSD) test was used to compare between treatment groups. Data were analyzed using SPSS version 25 statistical software.

Results and Discussion

Corn silk infusion (Zea mays L.)

The results of corn silk infusion (*Zea mays* L.) that have been obtained are then put into a freeze dryer for several days until a flat dry extract is formed thoroughly. This is done so that the compounds contained therein are stable because they will be stored for some time (Lestari et al., 2013; Oktavia et al., 2020). The resulting dry extract has a fainter color and a smoother texture compared to the extract before freeze-drying. Furthermore, it is stored in a tightly closed container.

Table 1. Phytochemical screening results

Secondary Metabolite Content	Repetition		
	I	II	III
Flavonoids	+	+	+
Phenols	-	-	-
Saponins	+	+	+
Alkaloids	+	+	+
Terpenoids (*Salkowski Test*)	-	-	-
Terpenoids and Steroids (*Liebermann Burchard Test*)	-	-	-

Note: Phytochemical screening of corn silk infusion (*Zea mays* L.) was carried out for three repetitions at the same time. (+) identified, (-) not identified.

In the phytochemical screening of corn silk infusion (*Zea mays* L.) with three repetitions at the same time, the results were obtained as in Table 1. Positive flavonoids are characterized by the onset of yellow color. Then the positive result of saponin is with the formation of foam about 15 minutes. The alkaloid test was identified by the formation of a white precipitate when given the Mayer reagent and a reddish-orange when given the Dragendorff reagent.

Phenol testing obtained negative results because the blue or purplish blue color did not appear, but a blackish color. Furthermore, the testing of terpenoid compounds (Salkowski test) obtained negative results because the reddish brown color between the faces of the layers was not formed, but yellow at the interface of the layers. Then the last test was terpenoids and steroids (Lieberman Burchard test) where the terpenoids obtained negative results because no red or purplish red color was formed but clear, on steroids also obtained negative results in the form of no green or bluish green color but clear.

Other parts of corn (*Zea mays* L.) plants such as corn leaves (*Zea mays* L.) have flavonoid compounds, saponins, and alkaloids that have potential as analgesics. The three compounds are also found in corn silk (*Zea mays* L.). Research conducted by Singh et al. (2022) on ethanol extract of corn silk (*Zea mays* L.) and research by Sholihah et al. (2012) on testing methanol extracts and decoct of corn silk (*Zea mays* L.) with flavonoid, saponin, and alkaloid compounds identified. Flavonoids, alkaloids, and saponins are known to play a role in the inhibition of the enzyme cyclooxygenase (COX) which can cause a decrease in the production of prostaglandins by arachidonic acid so that pain is reduced (Octavianus & Lolo, 2014; Tamimi et al., 2020; Wemay et al., 2013).

Analgesic effect of corn silk infusion (Zea mays) on rats

The results of the analgesic test of corn silk infusion (*Zea mays* L.) with the paw pressure test method using a sliding load analgesy meter on male white rats can be seen in **Figure 1** and **Table 2**. The graphs in Figure 1 and Table 2 show the average AUC pain threshold for each group at the 30th minute to the 240th minute.

Table 2. Average results of AUC pain threshold

Group	Mean ± SD
Positive Control (KP)	2558,23 ± 150,09[a]
Negative Control (KN)	1915,83 ± 46,65[b]
Treatment 1 (P1)	2207,08 ± 108,49[c]
Treatment 2 (P2)	2224,97 ± 122,97[c]
Treatment 3 (P3)	2407,50 ± 166.13[d]

Note: Different notations indicate significant differences (p<0.05).

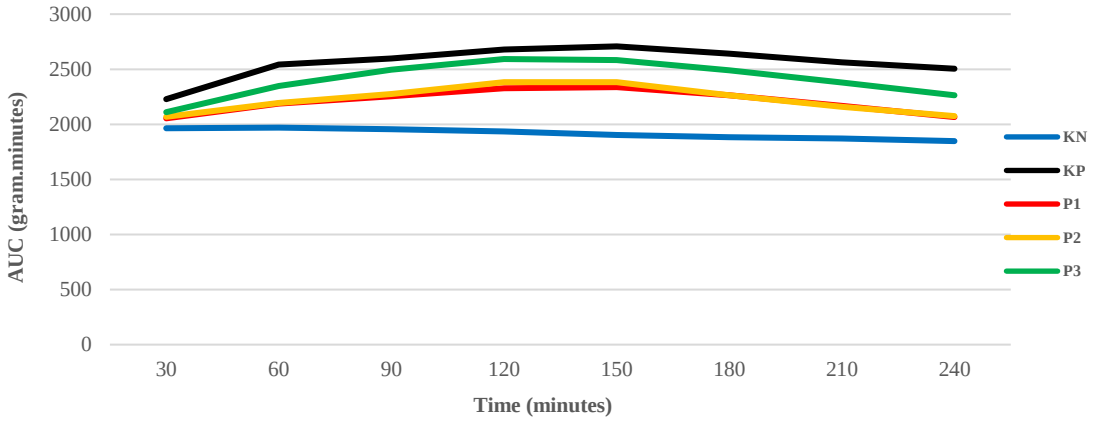

Figure 1. *Area Under Curve* (AUC) pain threshold chart

The KP group had a significant difference in pain threshold AUC against all groups with p<0.05. The average AUC of KP pain threshold was highest at the 150th minute, so it was found that the analgesic effect of KP reached a peak at the 150th minute. Mefenamic acid in humans reaches a peak at 2-4 hours. Factors such as species differences between test animals such as rats and humans can result in different metabolic processes (Octavianus & Lolo, 2014).

The P3 group had a significant difference in pain threshold AUC against all groups with p<0.05. The average AUC of the pain threshold of the P3 group was highest at the 120th minute. The graph in group P3 has the largest pain threshold AUC compared to P2 and P1. However, it is still below the KP group.

Group P2 has a significant difference in pain threshold AUC in almost all groups except group P1 with p=0.78. Furthermore, the P1 group had a significant difference in pain threshold AUC in almost all groups except the P2 group with p = 0.78. The existence of insignificant results in P1 with P2 is possible from the factor of low levels of active substances containing flavonoids, saponins, and alkaloids so that they have the same analgesic effect. It's just that in this study, phytochemical screening testing was not analyzed per dose group of corn silk infusion (*Zea mays* L.) because it was only analyzed qualitatively by knowing the color changes.

The KN group had a significant difference in pain threshold AUC against all groups with p<0.05. With the administration of distilled water, the pain threshold AUC was very low when compared to other groups. This can occur because distilled water does not contain active substances that can handle pain (Khotimah et al., 2021).

If the AUC value of the pain threshold produced is greater, it will produce a greater percentage of pain inhibition as well. The percentage of pain inhibition is the strength of a compound in the extract given to overcome the pain which can be seen in Figure 2.

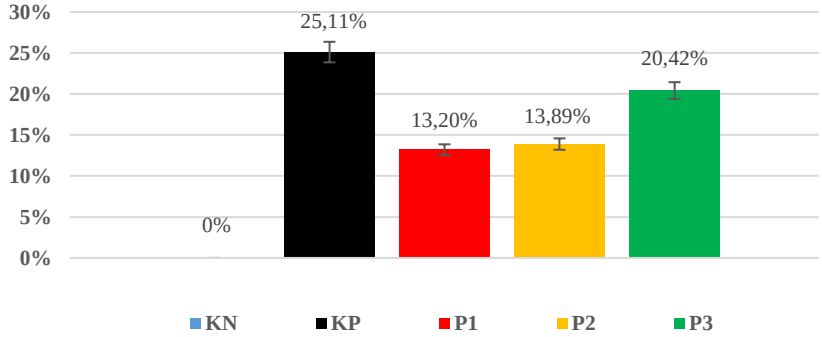

Figure 2. Histogram of pain inhibition percentage

The percentage of pain inhibition in the KP group with mefenamic acid suspension was obtained at 25.11%. One class of non-steroidal anti-inflammatory drugs (NSAIDs) that function to reduce pain is mefenamic acid. The mechanism of action is to inhibit the cyclooxygenase (COX) enzyme which causes less prostaglandin production so that pain can improve. Then from the administration of the three doses of infusion, the percentage of pain inhibition that is closest to the KP group is the P3 group, which is 20.42%. This shows that the better analgesic effect is when the amount of compound content that is absorbed is more. The percentage of pain inhibition produced by the P2 group is 13.89%, which indicates that the P2 group has less analgesic activity than the P3 group. The percentage of pain inhibition of the lowest infusion dose in the P1 group is 13.20% which indicates the weakest analgesic activity. In the KN group, the percentage of pain inhibition was 0% because distilled water does not contain compounds that can reduce pain.

Conclusion

a. In the phytochemical screening test of corn silk infusion (*Zea mays* L.), flavonoid, saponin, and alkaloid compounds were found.
b. A dose of 500 mg/kgBW of corn silk infusion (*Zea mays* L.) has the greatest analgesic effect on male white rats.

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