Volume 10, Issue 7, July – 2025
ISSN No: 2456-2165
International Journal of Innovative Science and Research Technology
https://doi.org/10.38124/ijisrt/25jul1591

Pharmacological Evaluation of the Analgesic Potential of *Eleusine indica* (Poaceae) Ethanolic Root Extract

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Publication Date: 2025/08/06

Abstract: Eleusine indica is a plant commonly seen at the roadsides in urban and rural areas. It is part of the Poaceae family wherein some of its plants are known to show analgesic activity. E. indica has been used as traditional medicine for inflammation and pain management. However, there are limited studies into the therapeutic activity and phytochemical profile of E. root extract. Thus, this study aims to conduct initial phytochemical screening on E. indica's root and evaluate its potential analgesic activity using the Hot Plate Method.

The roots were air-dried, blended, macerated with 70% ethanol, and filtered, and concentrated using a hot bath. The extracts were administered through oral gavage to the experimental group, while Ibuprofen (30 mg/kg) and distilled water (10 mL/kg) were administered for the control group. Analgesic activity was assessed using two methods: the hot plate test, which measured the latency of pain response, and the acetic acid-induced writhing test, which recorded the number of abdominal constrictions.

Acute oral toxicity testing was performed following OECD 423 guidelines, revealing no mortality or severe toxicity up to 5000 mg/kg, suggesting the extract is relatively non-toxic. In the writing test, both 500 mg/kg and 1000 mg/kg doses showed a significant reduction in writhes compared to the negative control ($p < 0.01$). In the hot plate test, the 500 mg/kg dose significantly increased pain response latency. Ibuprofen, as expected, have consistently shown significant analgesic effects in both tests. These findings suggest that E. indica root extract exhibits notable analgesic properties and could be a candidate for further development as a natural analgesic agent.

Keywords: *Eleusine indica, Poaceae, Ethanolic Extract, Analgesic Activity, Hot Plate Method, Acetic Acid Writhing Test.*

How to Cite: Renson M. Laraño; Crisel Faith A. Pacut; Jamalia A. Pangcatan; Sittie Amara U. Ansano; Patricia Mae B. Jarabe; Junnin Gay L. Garay (2025). Pharmacological Evaluation of the Analgesic Potential of *Eleusine indica* (Poaceae) Ethanolic Root Extract. *International Journal of Innovative Science and Research Technology*, 10(7), 3047-3061. https://doi.org/10.38124/ijisrt/25jul1591

I. INTRODUCTION

Pain has been present throughout our life, it has multiple and varied sensations that impact the quality of life. If you get injured, a neurotransmitter in the body will send a message to the brain which is the feeling of pain (Felman 2020). Analgesic drugs are available commercially that eases that pain, either by addressing the root cause of the pain or altering how your brain interprets it.

Analgesics are grouped into three types such as opioids, non-opioids, and compound analgesics (Ames, 2020). Non-opioids, such as nonsteroidal anti-inflammatory drugs analgesics, work at the peripheral nervous system by reducing inflammation at the site of injury. On the other hand, opioids act on the central nervous system by inhibiting the release of excitatory neurotransmitters in the brain (Ames, 2020; Reeves et al., 2022). Then, compound analgesics are the combination of anti-inflammatory agents and opioids which may result in enhanced efficacy.

ISSN No: 2456-2165 https://doi.org/10.38124/ijisrt/25jul1591

Poaceae family plants are known for their pharmacological properties such as anti-inflammatory activity (Gupta & Ranjan, 2020). Also, some species in the Poaceae family exhibit analgesic properties (Kassahun et al. 2020; Oluwatoyin et al. 2019; Ettebong et al. 2020). Eleusine indica, a plant from the Poaceae family, has been traditionally used to treat pain and inflammation in conditions like vaginal bleeding, fever, asthma, hemorrhoids, and flu-related symptoms (Zakri et al. 2021). Multiple researches on E. indica suggests that its leaves contain bioactive compounds with potential anti-inflammatory and pain-relieving effects, yet there are few related studies on its root.

The present study aims to investigate the analgesic potential of the ethanolic root extract of E. indica, focusing on its phytochemical profile, acute toxicity, and efficacy in established animal pain models.

➢ *Research Objectives*

This study aimed to determine the potential analgesic activity of the ethanolic root extract of *E. Indica,* and specifically to:

- Determine the percentage yield of the root extract of *E. indica*
- Determine the phytochemicals of the ethanolic root extract of *E. indica.*
- Determine the range of lethal dose of the ethanolic root extract of *E. indica* through acute oral toxicity testing.
- Compare the potential analgesic action of ethanolic root extract of *E. indica* with the controls Ibuprofen (positive) and Distilled water (negative).
- Determine which dosage of *E. indica* shows better potential analgesic activity.

➢ *Hypotheses*

- H_0: Mice administered with *E. indica* extract did not exhibit significant behavioral or physiological changes or death during the 14-day observation period.
- H_a: Mice administered with *E. indica* extract exhibit significant behavioral or physiological changes or death during the 14-day observation period.
- H_0: There was no significant difference in the latency of pain response in mice between the control group administered with Ibuprofen and the group administered with the ethanolic root extract of *E. indica* in the hot plate method.
- H_a: There was a significant difference in the latency of pain response in mice between the control group administered with Ibuprofen and the group administered with the ethanolic root extract of *E. indica* in the hot plate method.
- H_0: There was no significant difference in the number of writhing responses between the control group administered with Ibuprofen and the group administered with the ethanolic root extract of *E. indica* in the acetic acid induced writhing test.
- H_a: There was a significant difference in the number of writhing responses between the control group administered with Ibuprofen and the group administered with the ethanolic root extract of *E. indica* in the acetic acid induced writhing test.

II. MATERIALS AND METHODS

- *Collection and Authentication of E. Indica*

E. indica was collected at Brgy San Miguel Iligan City (8.237° N, 124.249° E). The whole plant was cleaned, and authenticated at a laboratory in MSU-IIT, Iligan City.

- *Preparation of the Plant Material*

First, the roots were soaked, then brushed, rinsed, and disinfected with 70% ethanol. The roots were then air-dried at room temperature (20-22 °C) for 3-4 weeks until it's brittle, then blended into powder and stored until extraction.

- *Extraction of the E. Indica Roots*

The powdered roots were soaked in 70% ethanol at 1:10 ratio of plant material (g) to solvent (ml). The mixture was then placed in an orbital shaker at 50 rpm for 5 days. The liquid was then filtered and evaporated using a hot bath at 48-50°C to remove the solvent, resulting in a viscous liquid extract with an amber or dark brown color.

- *Percentage Yield Determination*

The average percentage yield was calculated using the following formula:

$$Percentage\ yield = \left(\frac{Final\ weight}{Initial\ weight}\right) x\ 100$$

- *Phytochemical Screening*

The initial phytochemical screening was conducted at MSU-IIT, Iligan City, to identify the bioactive compounds present in the root, which could contribute to its potential analgesic activity.

➢ *Selection of Animals, Housing and Feeding Conditions*

Female Swiss albino mice (8 to 12 weeks old, weight variation only by ± 20%) were placed in a cage with proper ventilation, adequate space, and rice hulls as bedding to ensure the health and wellbeing of the mice. The room was maintained under a 12-hour light/dark cycle at 22± 3ºC, and 30-70% humidity. The mice were fasted for 3-4 hours before the acute toxicity test and experiment.

➢ *Acute Toxicity Test*

The mice were randomly selected and divided into 5 groups of 3. Following OECD 423, each mice received a single oral dose of 300, 2000, and 5000 mg/kg of extract and were observed for 14 days for signs of toxicity or mortality. At the end, all mice underwent a gross necropsy. The mice were dissected, and their organs were examined for any signs of toxicity such as organ damage or abnormalities.

➢ *Therapeutic Evaluation Preparation*

The mice were randomly grouped into 5 groups of 5. The mice were fasted for 3-4 hours before each experiment was conducted. Their body weight was recorded before the

ISSN No: 2456-2165 https://doi.org/10.38124/ijisrt/25jul1591

experiment to calculate the specific volume to be administered on each mice.

➢ *Preparation of Control and Experimental Solutions*

- *Preparation of Negative Control*

Distilled water at 10 mL/kg according to their bodyweight was administered orally as the negative control, providing a baseline reference for evaluating the potential analgesic activity of the ethanolic root extract of E. indica.

- *Preparation of Positive Control*

The ibuprofen at 30 mg/kg was prepared as a 0.3% w/v solution using a solvent composed of 90% distilled water, 5% ethanol to dissolve the powder, and 5 %NaOH to prevent precipitation.

- *Preparation of Experimental*

The ethanolic extract doses per group were set at 1/20th (low dose), 1/10th (mid dose), and 1/5th (high dose) of the LD50, administered at 10 mL/kg.

➢ *Acetic Acid Writhing Test*

The mice receive their corresponding treatments according to their dosage group then 0.7% acetic acid solution is administered intraperitoneally at 10 mL/kg after 30 minutes. The mice were then observed individually for 30 mins for any types of writhing (mild or intense) and other types of behaviours.

To quantify the analgesic effect, the %inhibition of writhing was then calculated to provide a standardized comparison of the extract's efficacy in reducing writhing behavior. The formula used is as follow as described by (Cheng et al. 2016):

$$\%Inhibition = \frac{[No.of\ writhes\ (negative) - No.of\ writhes\ (treatment)]}{No.of\ writhes\ (negative)} x100$$

➢ *Hot Plate Method*

The hotplate was set at 55°C which was sufficiently painful to elicit a response from the animal while being safe enough to avoid irreversible harm. Baseline latency was recorded prior to treatment. Post-treatment datas were then recorded at 30, 60, 90, and 120 minutes, by placing the mice back on the hotplate. A longer latency indicating an analgesic activity.

The Maximum Possible Effect %MPE was then calculated to provide a standardized measure of the analgesic effect using the formula described by (Akah & Ezeugo 2020):

$$\%MPE = \frac{Post\ Latency - Pre\ Latency}{Cutoff - Pre\ Latency} x100$$

III. RESULTS AND DISCUSSIONS

➢ *Percentage Yield*

The average percentage yield of concentrated extract of E. indica was 1.97% with 0.15% standard deviation. While a 1.97% yield is relatively low, the ±0.15% variation across the batches indicates that the extraction method was consistent, reproducible, and reliable under the same conditions.

➢ *Phytochemical Screening*

The phytochemical screening was conducted at the Department of Chemistry in Mindanao State University–Iligan Institute of Technology (MSU-IIT). The results indicated the presence of bioactive compounds in the ethanolic root extract of Eleusine indica as shown in Table 2. The screening revealed the presence of flavonoids, phenols, saponins, alkaloids, tannins, steroids, and terpenoids.

Table 1 Average Percentage Yield of Ethanolic Root Extract of *E. Indica*

Batch	Plant (initial)	Extract (Final)	$\%yield\ (\frac{Final\ weight}{Initial\ weight})x\ 100$	Average Yields
1	100g	1.8g	$(\frac{1.8g}{100g})x\ 100\ =\ 1.8\%$	$\left(\frac{1.8+2.1+2}{3}\right)$=1.97% ± 0.15%
2	100g	2.1g	$(\frac{2.1g}{100g})x\ 100\ =\ 2.1\%$	
3	100g	2g	$(\frac{2g}{100g})x\ 100\ =\ 2\%$	

Table 2 Phytochemicals Present of Ethanolic Root Extract of *E. Indica*

Plant Sample	Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Steroids	Terpenoids
Eleusine indica	+	+++	+++	+++	+	+	+

Legends: (+): Weakly positive; (++): Moderately positive; (+++): Strongly positive

➢ *OECD 423*

During the acute toxicity assessment, hunched posture, laboured respiration, and lethargy were observed in all treatment groups within the first 1 to 2 hours post-administration, however, no mortality was recorded at doses of 300 mg/kg, 2000 mg/kg, or 5000 mg/kg throughout the 14-day observation period. Based on these findings, the median

International Journal of Innovative Science and Research Technology
https://doi.org/10.38124/ijisrt/25jul1591

lethal dose (LD_{50}) of the extract is estimated to be greater than 5000 mg/kg, indicating a low level of acute oral toxicity.

➢ *Acetic Acid-Induced Method*

The descriptive data from Batch 1 of the acetic acid writhing test demonstrated the potential analgesic effects of the ethanolic root extract of Eleusine indica at varying concentrations (250 mg/kg, 500 mg/kg, and 1000 mg/kg), compared to a positive control (Ibuprofen at 30 mg/kg) and a negative control (Distilled Water at 10 mL/kg).

In Batch 1, the negative control group exhibited the highest mean number of writhing responses at 24.8 (±3.42), indicating no analgesic activity, with a 0% inhibition rate. In contrast, the positive control showed the lowest mean at 11.6 (±1.82), with a 53.3% inhibition rate, confirming the effectiveness of ibuprofen.

Among the extract-treated groups, the 1000 mg/kg dose produced the lowest mean number of writhes (18.6 ± 5.32), with an inhibition of 24.8%, followed by 250 mg/kg (19.6 ± 4.77 and 20.97% inhibition) and 500 mg/kg (20.4 ± 3.05 and 17,74% inhibition), suggesting a modest, dose-responsive analgesic effect.

In Batch 2, the negative control group again showed the highest writhing mean at 23.6 (±2.20), with 0% inhibition rate, while the positive control group had a reduced response of 14.4 (±3.85), with 38.14% inhibition rate. The extract-treated groups exhibited a clearer dose-dependent trend in this batch, with the 1000 mg/kg group showing the lowest mean writhes (15.2 ± 2.68) and a 35.14% inhibition, followed by 500 mg/kg (17.6 ± 4.62 and 25.42% inhibition) and 250 mg/kg (21 ± 5.39 and 10.17% inhibition), indicating a slightly stronger analgesic effect compared to Batch 1.

Overall, both batches indicated moderate analgesic potential for E. indica, particularly at higher doses. The consistency of reduced writhing behaviors across both batches supported the reliability of the extract's efficacy. Additional analysis and repeated trials are needed to substantiate these findings and to better understand the underlying mechanisms of the extract's pharmacological activity.

Table 3 Batch 1 Result of the Acetic Acid Writhing Test with Mean and Standard Deviation and the Average Inhibition Rate Per Group

Batch 1 (original)	**Number of Writhing**		**Average Inhibition Rate %**
	Mean	**Standard deviation**	
Group 1: 250 mg/kg	19.6	4.7749	20.97%
Group 2: 500 mg/kg	20.4	3.05	17.74%
Group 3: 1000 mg/kg	18.6	5.32	24.8%
Positive Control	11.6	1.817	53.3%
Negative Control	24.8	3.42	0%

Table 4 Batch 2 Result of the Acetic Acid Writhing Test with Mean and Standard Deviation and the Average Inhibition Rate Per Group

Batch 2 (replicate)	**Number of Writhing**		**Average Inhibition Rate %**
	Mean	**Standard deviation**	
Group 1: 250 mg/kg	21	5.385	10.17%
Group 2: 500 mg/kg	17.6	4.62	25.42%
Group 3: 1000 mg/kg	15.2	2.68	35.14%
Positive Control	14.4	3.85	38.14%
Negative Control	23.6	2.2	0%

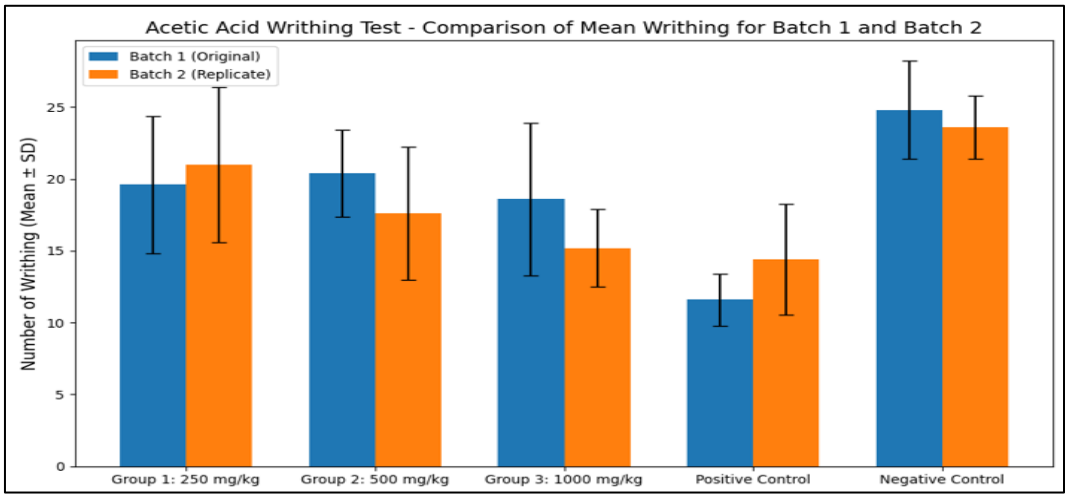

Fig 1 Bar Graph Result of Batch 1 and 2 for Acetic Acid Writhing Test

ISSN No: 2456-2165 https://doi.org/10.38124/ijisrt/25jul1591

- *Statistical Analysis*

✓ *Anova*

In Table 5, an Analysis of Variance (ANOVA) was done on the Batch 1 to determine whether the ethanolic root extract of Eleusine indica had a significant analgesic effect compared to the control groups. The analysis focused on the number of writhing responses in mice, with a significance level set at $p < 0.05$ (significant) and $p < 0.01$ (highly significant).

The ANOVA result for the number of writhing yielded an F-value of 7.533 and a p-value of 0.0007159, which is highly significant ($p < 0.01$). This indicates a statistically significant difference among the treatment groups in terms of writhing responses, suggesting that the E. indica extract, particularly at certain concentrations, effectively reduced the number of abdominal constrictions caused by acetic acid. These findings support the hypothesis that E. indica possesses peripheral analgesic properties, as the acetic acid writhing test is known to reflect pain mediated by peripheral mechanisms.

For Batch 2, an Analysis of Variance (ANOVA) was performed to assess the significant differences in both the number of writhing behaviors across the control groups (negative and positive) and the different concentrations of Eleusine indica ethanolic root extract (250 mg/kg, 500 mg/kg, and 1000 mg/kg).

The ANOVA result for number of writhing produced an f-value of 4.91828 and a p-value of 0.006316, which is statistically significant ($p < 0.01$). This indicates that there is a significant difference among the treatment groups in terms of the number of writhing responses. This suggests that the ethanolic root extract of E. indica, especially at higher concentrations, effectively reduces the number of abdominal writhes induced by acetic acid, further supporting its potential analgesic activity in the peripheral pain pathway.

Table 5 ANOVA Results (Batch 1): Writhing Count – Control vs. E. Indica (250–1000 mg/kg)

ANOVA Result of Batch 1 (original)	f-value	p-value
Number of Writhing	7.533	0.0007159**

Note: Significant if p-value < 0.05* and p-value < 0.01**

Table 6 ANOVA Results (Batch 2): Writhing Count – Control vs. E. Indica (250–1000 mg/kg)

ANOVA Result of Batch 2 (replicate)	f-value	p-value
Number of Writhing	4.91828	0.006316**

Note: Significant if p-value < 0.05* and p-value < 0.01**

- *Tukey's HSD (Post HOC)*

The Tukey's HSD test results for the number of writhing in Batch 1 and Batch 2 provided insights into the specific significant differences between the control groups (Negative and Positive) and the different concentrations of the ethanolic root extract of Eleusine indica (250 mg/kg, 500 mg/kg, and 1000 mg/kg).

In Batch 1, the 250 mg/kg dose showed a significant reduction in the number of writhing compared to the positive control (Ibuprofen) with a p-value of 0.0081, suggesting a potential analgesic effect. The 500 mg/kg dose exhibited a highly significant difference ($p = 0.0005$) compared to the positive control, indicating that it was more effective than Ibuprofen in reducing the number of writhing responses.

Additionally, the 500 mg/kg dose also showed a significant difference ($p = 0.0304$) when compared to the negative control, further confirming its analgesic potential. For the 1000 mg/kg dose, a significant difference ($p = 0.0238$) was observed compared to the positive control, indicating that the highest dose of the extract also produced observable analgesic effects. Moreover, the difference between the positive control and the negative control was highly significant ($p = 0.00001$), reaffirming the effectiveness of Ibuprofen in reducing pain-related behaviors.

In Batch 2, the 250 mg/kg dose did not show a significant difference when compared to the positive control ($p = 0.0563$), suggesting that the analgesic effect at this dose was less pronounced in this batch. However, the 500 mg/kg dose exhibited a significant difference ($p = 0.0304$) compared to the negative control, indicating that the extract at this concentration effectively reduced the number of writhing behaviors. The 1000 mg/kg dose showed a highly significant difference ($p = 0.00063$) compared to the negative control, further supporting its potent analgesic activity at this dose. Finally, the positive control exhibited a highly significant difference ($p = 0.0017$) when compared to the negative control, confirming the reliability of Ibuprofen as a potent analgesic.

In summary, Tukey's HSD test results demonstrated that higher doses of E. indica (particularly 500 mg/kg and 1000 mg/kg) were effective in reducing the number of writhing behaviors, especially in Batch 1, with significant reductions observed compared to both control groups. These findings suggest that E. indica has observable analgesic potential, particularly at the higher concentrations tested.

Table 7 Tukey's HSD Analysis (Batches 1 & 2): Writhing – Control vs. E. Indica (250–1000 mg/kg)

Post hoc test result	Batch 1	Batch 2
	p-value	p-value
250 mg/kg vs 500 mg/kg	0.760283519	0.315006206
250 mg/kg vs 1000 mg/kg	0.762427253	0.063225952

250 mg/kg vs Positive Control	0.008061284**	0.056302837
250 mg/kg vs negative control	0.08309935	0.346593507
500 mg/kg vs 1000 mg/kg	0.529995101	0.344210179
500 mg/kg vs positive control	0.000545129**	0.267820101
500 mg/kg vs negative control	0.064075992	0.030360132*
1000 mg/kg vs positive control	0.023760842*	0.712847273
1000 mg/kg vs negative control	0.059731538	0.000629168**
Positive Control vs negative control	0.00001**	0.001651748**

Note: Significant if p-value < 0.05* and p-value < 0.01**

- *Paired T tests*

The Paired t-test results, as shown in Table 8, were used to assess the significant differences between Batch 1 and Batch 2 for the number of writhing behaviors observed in the acetic acid writhing test at various concentrations of Eleusine indica ethanolic root extract (250 mg/kg, 500 mg/kg, and 1000 mg/kg), as well as the control groups (Negative and Positive).

For the number of writhing, the p-values for all treatment groups—250 mg/kg (p = 0.675), 500 mg/kg (p = 0.29), and 1000 mg/kg (p = 0.2377)—indicated no significant differences between Batch 1 and Batch 2. This suggests that the writhing response in these groups was consistent across both batches, demonstrating stable results for the ethanolic root extract in both trials. Additionally, the writhing behavior for the Positive Control group (Ibuprofen) also showed no significant difference (p = 0.179) between the batches, indicating consistency in the analgesic effect of Ibuprofen across batches. The Negative Control group had a p-value of 0.527, suggesting no significant variation in writhing behavior between the two batches for the distilled water treatment.

In summary, the writhing response showed consistency between Batch 1 and Batch 2 for both the ethanolic root extract of Eleusine indica and the control groups. This suggests that the analgesic effect, as measured by writhing behavior, was stable across batches.

Table 8 Paired T-Test (Batches 1 & 2): writhing Count – Control vs. E. Indica (250–1000 mg/kg)

Paired t-test result of Batch 1 and 2	**Number of Writhing**
	p-value
Group 1: 250 mg/kg	0.675
Group 2: 500 mg/kg	0.29
Group 3: 1000 mg/kg	0.2377
Positive Control	0.179
Negative Control	0.527

Note: Significant if p-value < 0.05* and p-value < 0.01**

➢ *Hotplate Test*

The hotplate method results from Batch 1 provide further evidence of the analgesic potential of the ethanolic root extract of Eleusine indica, as measured by the reaction latency to thermal pain. Pre-treatment latency values were relatively similar across all groups, establishing a baseline for comparison.

In Batch 1, the negative control group (Distilled Water) showed consistently low latency values (from 9.4s to 14s) with an MPE ranging from -3.88% at 30 minutes to 22.33% at 120 minutes, reflecting a slight improvement, but still far from the analgesic effects seen in the treatment groups. In contrast, the positive control group (Ibuprofen 30 mg/kg) demonstrated a clear and progressive increase in latency, particularly from 60 to 120 minutes (peaking at 24.2s), and MPE reaching 68.48% at 120 minutes confirming its expected analgesic effect.

The 1000 mg/kg group showed the most remarkable effect, with latency increasing from 10s pre-treatment to 29.6s at 120 minutes, corresponding to a peak MPE of 98%. The 500 mg/kg group also showed a significant effect, with latency reaching 22.6s at 120 minutes and an MPE of 56.47%. The 250 mg/kg group exhibited moderate analgesic effects, with latency rising to 22s and an MPE of 64.91% at 120 minutes, despite fluctuations at 60 minutes.

Interestingly, in batch 2, the Positive control group showed only a modest increase in latency over time, with an MPE ranging from 0% to 31.73%. While this demonstrates some analgesic effect, the response was not as pronounced as observed in Batch 1. The Negative control group showed a small increase in latency from 15.2 seconds to 16.0 seconds, with an MPE from -12.16% to 5.41%. Among the E. indica treatment groups, the 500 mg/kg dose produced the most consistent analgesic effect, with latency increasing from 14.4 seconds at baseline to 20.2 seconds at 120 minutes and MPE of 41.18%. This pattern indicates a moderate and sustained analgesic effect. The 1000 mg/kg group however, showed a lesser effect compared to batch 1, with latency reaching 18.0 seconds at 120 minutes and an MPE of 41.18%. The 250 mg/kg group showed little change in latency across time points(14-15 seconds), with a low MPE of 9.3% at 30 minutes and 37.18% at 120 minutes, indicating limited analgesic action at this dose.

Overall, the results from both batches support the analgesic potential of E. indica root extract. Extract-treated groups consistently exhibited longer reaction latencies compared to the negative control, particularly at higher doses

and longer time points. However, some variations in responses across doses and batches were observed, likely due to individual biological differences or extract variability. The results from the 500 mg/kg and 1000 mg/kg batch supports a dose-dependent analgesic effect. These results align well with those from the acetic acid writhing test, further supporting the extract's efficacy and suggesting that Eleusine indica may exert both peripheral analgesic effects.

Supporting this, a study by Akah and Ezeugo (2020) evaluated the anti-nociceptive effects of Eleusine indica in rodents. The research demonstrated that both extracts exhibited significant dose-dependent analgesic activity in animal models, suggesting that E. indica possesses compounds capable of modulating pain perception. Although their study focused on leaf extracts, the observed effects align with the current findings, reinforcing the potential of E. indica roots as a natural source of analgesic agents.

Table 9 Maximum Possible Effect for Hot Plate Test - Batch 1 and 2

	Batch 1				Batch 2			
Group	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Group 1: 250 mg/kg	22.81%	-5.26%	12.28%	64.91%	9.3%	12.79%	16.28%	9.3%
Group 2: 500 mg/kg	43.53%	1.18%	55.29%	56.47%	-10.26%	24.36%	41.03%	37.18%
Group 3: 1000 mg/kg	-6%	37%	12%	98%	25.49%	39.22%	32.35%	41.18%
Positive Control	-18.48%	22.83%	46.74%	68.48%	0%	6.73%	18.27%	31.73%
Negative Control	-3.88%	10.68%	24.27%	22.33%	-12.16%	-9.46%	17.57%	5.41%

Table 10 Batch 1 Result for Hotplate Method with Mean and Standard Deviation

Batch 1 (Original)	**Pre-treatment**	**30 mins**	**60 mins**	**90 mins**	**120 mins**
Group 1: 250 mg/kg	7.2 ± 3.35	12.4 ± 4.83	6.0 ± 1.00	10.0 ± 4.90	22.0 ± 6.50
Group 2: 500 mg/kg	13.0 ± 1.40	20.4 ± 3.85	13.2 ± 10.50	22.4 ± 7.13	22.6 ± 10.85
Group 3: 1000 mg/kg	10.0 ± 2.74	8.8 ± 4.40	17.4 ± 3.90	12.4 ± 3.50	29.6 ± 0.89
Positive Control	11.6 ± 5.64	8.2 ± 2.39	15.8 ± 4.32	20.2 ± 7.46	24.2 ± 8.30
Negative Control	9.4 ± 7.09	8.6 ± 3.78	11.6 ± 1.51	14.4 ± 9.29	14.0 ± 6.96

Table 11 Batch 2 Result for Hotplate Method with Mean and Standard Deviation

Batch 2 (replicate)	**Pre-treatment**	**30 mins**	**60 mins**	**90 mins**	**120 mins**
Group 1: 250 mg/kg	12.8 ± 6.38	14.4 ± 3.13	15.0 ± 5.29	15.6 ± 3.97	14.4 ± 5.27
Group 2: 500 mg/kg	14.4 ± 7.16	12.8 ± 3.11	18.2 ± 4.10	20.8 ± 5.02	20.2 ± 5.85
Group 3: 1000 mg/kg	9.6 ± 3.65	14.8 ± 4.82	17.6 ± 7.02	16.2 ± 5.89	18.0 ± 3.24
Positive Control	9.2 ± 4.02	9.2 ± 3.11	10.6 ± 3.58	13.0 ± 3.39	15.8 ± 3.90
Negative Control	15.2 ± 5.40	13.4 ± 3.78	13.8 ± 2.05	17.8 ± 5.40	16.0 ± 2.35

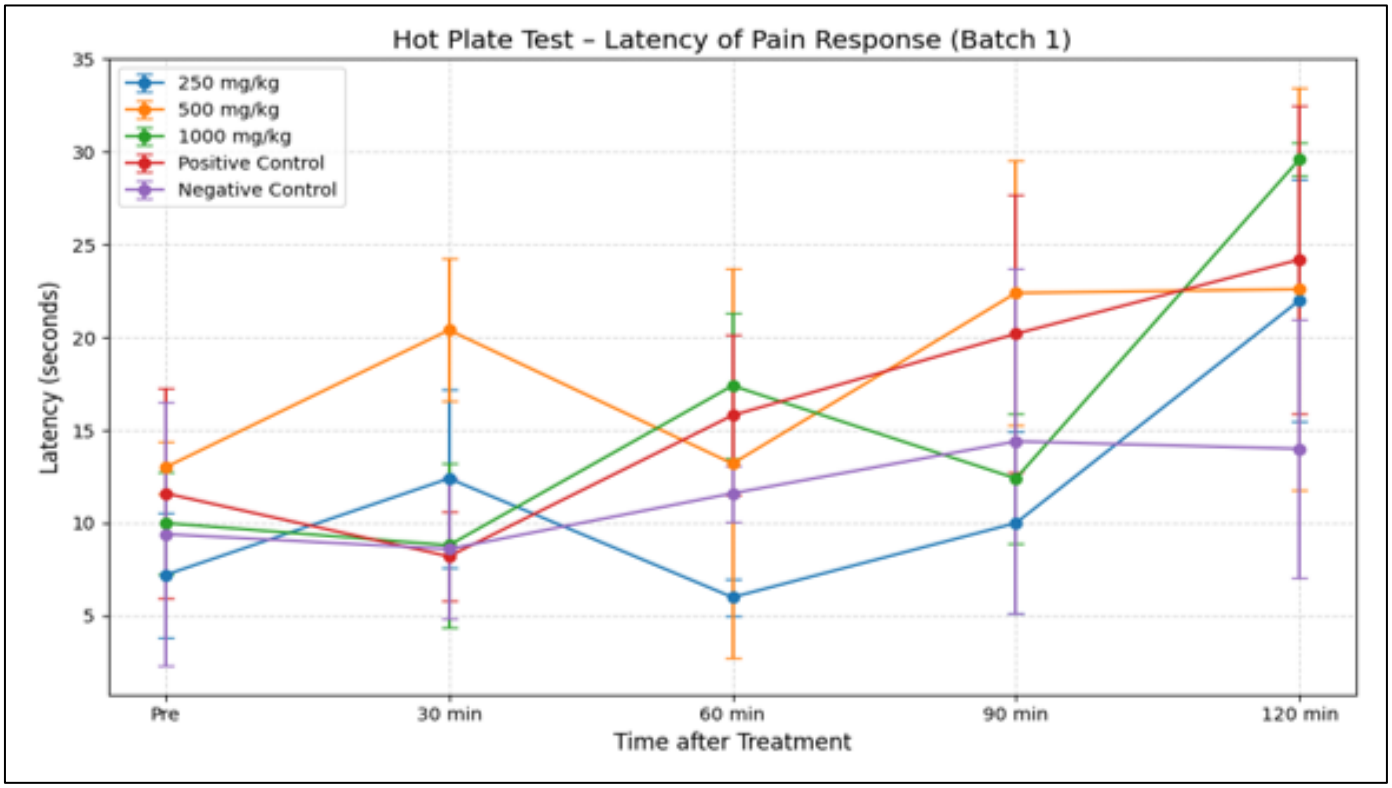

Fig 2 Line Graph for the Batch 1 Result of Hotplate Method

ISSN No: 2456-2165 https://doi.org/10.38124/ijisrt/25jul1591

- *Analysis*

✓ *Anova*

The researchers used Analysis of Variance (ANOVA) to test for significant differences between the control group administered Ibuprofen and the group administered the ethanolic root extract of E. indica in the mice's latency of pain response, measured by the hot plate method with a significance level set at $p < 0.05$ (significant) and $p < 0.01$ (highly significant).

In Table 12, to statistically evaluate the effectiveness of the ethanolic root extract of Eleusine indica compared to both the positive (Ibuprofen) and negative (Distilled Water) controls, the researchers applied a one-way Analysis of Variance (ANOVA) to the hotplate test results from both Batch 1 and Batch 2. This method was used to determine whether there were any statistically significant differences in the latency of pain response among the different treatment groups, with a significance level set at $p < 0.05$ (significant) and $p < 0.01$ (highly significant).

In Batch 1, the computed f-value was 1.2 with a corresponding p-value of 0.34. Since the p-value is greater than 0.05, this result indicates that there is no statistically significant difference in the pain latency response among the treatment groups in Batch 1. This suggests that, although variations in latency were observed descriptively, these differences are likely due to random variation rather than a true effect of the treatments.

However, in Batch 2, the ANOVA yielded an f-value of 2.95 with a p-value of 0.0455, which is less than 0.05, indicating a statistically significant difference among the groups. This suggests that in the replicate trial, at least one of the treatment groups (most likely the 500 mg/kg or 1000 mg/kg E. indica group) demonstrated a pain latency response that significantly differed from the others, particularly the controls. This result strengthens the claim that E. indica may have dose-dependent analgesic properties, as the extract showed a measurable and statistically significant effect in Batch 2.

Overall, while Batch 1 did not show significant statistical evidence, the findings from Batch 2 support the potential analgesic activity of Eleusine indica extract, which aligns with the patterns observed in the descriptive data.

Table 12 ANOVA Results: Hotplate Test (Batch 1 & 2) – Control vs. E. Indica Extract

ANOVA Result	f-value	p-value
Batch 1	1.2	0.34
Batch 2	2.95	0.0455*

Note: Significant if p-value < 0.05* and p-value < 0.01**

- *Tukey's HSD (Post HOC)*

The Tukey's HSD test for Batch 2 was conducted to determine which specific treatment pairs showed statistically significant differences in their effects on pain latency in mice. Among the various pairwise comparisons, two comparisons yielded statistically significant results at the 0.05 level.

First, the 500 mg/kg E. indica extract group showed a significant difference when compared to the positive control group (Ibuprofen), with a

p-value of 0.022. This suggests that the analgesic effect observed in the 500 mg/kg group was significantly stronger than that of the standard drug, highlighting the potential of E. indica at this concentration as a promising natural analgesic.

Second, a significant difference was also found between the positive control and the negative control groups, with a p-value of 0.039. This result confirms the effectiveness of Ibuprofen in increasing pain latency compared to distilled water, thus validating the experimental model.

Other comparisons, including those between extract concentrations (250 mg/kg vs. 500 mg/kg or 1000 mg/kg) and between extract groups and the negative control, did not reach statistical significance. This suggests that the differences in analgesic effects could be attributed to random variability or that they may not be strong enough to be distinguished statistically.

Overall, Tukey's HSD results reinforce the findings from the ANOVA in Batch 2, particularly highlighting that the 500 mg/kg concentration of Eleusine indica extract may offer a more potent analgesic effect than Ibuprofen under the conditions tested.

Table 13 Tukey's HSD Analysis: Batch 2 Hotplate Method – Control vs. E. Indica Extract (250, 500, 1000 mg/kg)

Post hoc test result for Batch 2	p-value
250 mg/kg vs 500 mg/kg	0.123607123
250 mg/kg vs 1000 mg/kg	0.628092109
250 mg/kg vs Positive Control	0.065494138
250 mg/kg vs negative control	0.410079726
500 mg/kg vs 1000 mg/kg	0.379595005
500 mg/kg vs positive control	0.022466819*
500 mg/kg vs negative control	0.282592863
1000 mg/kg vs positive control	0.099812427

ISSN No: 2456-2165 https://doi.org/10.38124/ijisrt/25jul1591

1000 mg/kg vs negative control	1
Positive Control vs negative control	0.039230443*

Note: Significant if p-value < 0.05* and p-value < 0.01**

- *Paired T Test*

Tables 14 and 15 present the Paired t-test results comparing the pre-treatment latency to the latency at 30, 60, 90, and 120 minutes after treatment using the hotplate method. The goal of this analysis is to determine if there is a significant change in the mice's pain response (latency) over time, with longer latency indicating an analgesic effect for Batch 1.

In Batch 1, no significant difference was found at 30 minutes (p = 0.157) or 60 minutes (p = 0.509). However, a significant increase in latency was observed at 120 minutes (p = 0.019), suggesting some analgesic activity over a longer period. Similarly, in Batch 2, there were no significant effects at 30, 60, or 90 minutes, but a significant effect was observed at 120 minutes (p = 0.0307), indicating potential analgesic activity at the later time point. The findings from both batches suggest that the 250 mg/kg dose of the ethanolic root extract of Eleusine indica exhibits weak but delayed analgesic activity, with consistent significance appearing only at 120 minutes post-treatment.

In Batch 1, a significant increase in latency was observed at 30 minutes (p = 0.013) and 90 minutes (p = 0.038), suggesting that this concentration may provide a quicker analgesic response. However, no significant effect was noted at 60 minutes (p = 0.970) or 120 minutes (p = 0.121). In contrast, Batch 2 results showed no significant effects at 30, 60, or 90 minutes. However, a significant effect was observed at 120 minutes (p = 0.0032), indicating that the 500 mg/kg dose had an effect at the later time point, suggesting delayed analgesic action.

Overall, the 500 mg/kg dose exhibited moderate analgesic potential, with Batch 1 indicating a faster onset and Batch 2 highlighting effects more prominent at later time points. These findings point to a variable but present analgesic activity at this concentration.

For the 1000 mg/kg group in Batch 1, there was a significant increase in latency at 60 minutes (p = 0.022) and a highly significant increase at 120 minutes (p = 0.0001), indicating a strong and progressive analgesic response over time. In Batch 2, a borderline effect was observed at 60 minutes (p = 0.0647), while a significant effect was again confirmed at 120 minutes (p = 0.0032). These results suggest that the 1000 mg/kg dose consistently exhibits the strongest analgesic activity among all groups, particularly at the 120-minute mark, with both batches supporting its efficacy.

Ibuprofen, used as the positive control, showed significant analgesic effects at 90 minutes in Batch 1 (p = 0.039) and at 120 minutes in Batch 2 (p = 0.0048), supporting its expected analgesic activity. These results align with its known pharmacological profile and validate the experimental setup by confirming the expected analgesic effects at later time intervals.

In the Negative control group, distilled water did not show any significant effect at 30 minutes (p = 0.688) or 60 minutes (p = 0.533) in Batch 1. However, it surprisingly exhibited significant effects at 90 minutes (p = 0.019) and 120 minutes (p = 0.0036) in Batch 1. In Batch 2, while the effects were relatively weak compared to the active treatments, a significant result was observed at the 90-minute time point (p = 0.025), with other time points showing no significance. These mild and inconsistent effects may be due to natural variability or spontaneous behavior rather than any true analgesic action.

Table 14 Paired T-Test (Batch 1): Hotplate Pre-Treatment vs. Time Points – Control vs. E. Indica (250–1000 mg/kg)

Paired t-test result Batch 1 (original)	**Group 1: 250 mg/kg**	**Group 2: 500 mg/kg**	**Group 3: 1000 mg/kg**	**Positive Control**	**Negative Control**
	p-value	**p-value**	**p-value**	**p-value**	**p-value**
Pre-treatment vs 30mins	0.156995743	0.012633993*	0.667944706	0.366342274	0.68845709
Pre-treatment vs 60 mins	0.508621407	0.969668589	0.022196264*	0.345340772	0.533320333
Pre-treatment vs 90 mins	0.442991973	0.038395565*	0.391761908	0.038810211*	0.018541393*
Pre-treatment vs 120 mins	0.019250042*	0.121383775	0.0001**	0.105221251	0.003552295**

Note: Significant if p-value < 0.05* and p-value < 0.01**

Table 15 Paired T-Test (Batch 2): Hotplate Pre-Treatment vs. Time Points – Control vs. E. Indica (250–1000 mg/kg)

Paired t-test result Batch 2 (replicate)	**Group 1: 250 mg/kg**	**Group 2: 500 mg/kg**	**Group 3: 1000 mg/kg**	**Positive Control**	**Negative Control**
	p-value	**p-value**	**p-value**	**p-value**	**p-value**
Pre-treatment vs 30mins	0.626833362	0.444201673	0.099804655	1	0.265847286
Pre-treatment vs 60 mins	0.53880062	0.219890393	0.064676894	0.107938822	0.487572915
Pre-treatment vs 90 mins	0.415924717	0.148870868	0.057707566	0.087172403	0.025481481*
Pre-treatment vs 120 mins	0.691557857	0.030698563*	0.003184813**	0.004804665**	0.692571071

Note: Significant if p-value < 0.05* and p-value < 0.01**

IV. CONCLUSION

The study confirms that the ethanolic root extract of Eleusine indica exhibits significant analgesic activity, with its effects increasing with dosage, particularly at the 500 mg/kg and 1000 mg/kg dose, which provided the most effective pain relief. The extract was also found to be safe, with no toxic effects observed even at the high dose of 5000 mg/kg. Its effectiveness, especially when compared to ibuprofen in the acetic induced and hot plate test, suggests that it may exert peripheral analgesic effects. However, the variability observed between batches, particularly in pain latency (hotplate method), indicates the need for further investigation into the extract's consistency and the mechanisms underlying its action. These results support the traditional use of E. indica for pain management and highlight its potential as a natural analgesic.

➢ *Recommendations*

- Further research on the analgesic activity of Eleusine indica is recommended, particularly on other kinds of pain models to validate and expand our findings.
- Use various extraction methods to determine which technique provides the highest yield as identifying the most effective method will help optimize both the quantity and quality of bioactive compounds for pain relief research.
- It is recommended that this study is replicated but with more batches for further validation and support.
- To compare the analgesic activity of E. indica to other plants of the Poaceae family. This will help determine whether other species in the family exhibit similar or stronger pain-relieving properties, thereby providing a broader understanding of the therapeutic potential of the Poaceae family in pain management.

ACKNOWLEDGMENT

We sincerely thank everyone who played a part in the completion of this study. Above all, we offer our heartfelt gratitude to the **Almighty Father** for His constant guidance, endless mercy, and the hope that sustained us throughout this journey.

Our deepest appreciation goes to our academic mentors—**Ms. Patricia Mae B. Jarabe, RPh**, our devoted thesis adviser, for her invaluable guidance and support, and **Ms. Junnin Gay L. Garay, RPh, CPh, MS Pharm**, our Research and Thesis Writing professor, for her insightful feedback and academic expertise.

We sincerely thank **Adventist Medical Center College** for equipping us with the vital support and resources that made this research possible.

Lastly, we are truly thankful to our f**amilies and loved ones**, whose unwavering support and encouragement played a vital role in bringing this study to a successful conclusion.

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ISSN No: 2456-2165 https://doi.org/10.38124/ijisrt/25jul1591

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