Wiley
Advances in Pharmacological and Pharmaceutical Sciences
Volume 2025, Article ID 4647835, 11 pages
https://doi.org/10.1155/adpp/4647835

WILEY

Research Article

Pain-Relieving Property of *Artemisia abyssinica* and *Lepidium sativum* (Essential Oil) in Mice

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Received 22 March 2025; Revised 27 June 2025; Accepted 7 July 2025

Academic Editor: Benedetto Natalini

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Background: Ethiopian traditional medicine uses *Artemisia abyssinica* and *Lepidium sativum* to treat headaches and other pain-related disorders. Nevertheless, this assertion has not yet received enough scientific support. This study looked into the analgesic effects of *A. abyssinica* and *L. sativum* essential oils in mice.
Methods: The extraction was carried out via conventional methods. Essential oils were extracted by hydrodistilling fresh seeds and aerial parts of *A. abyssinica* and *L. sativum*, respectively. Test doses for both plants were 100, 200, and 400 mg/kg b.w. Using a mouse model of acetic acid-induced writhing, the peripheral analgesic efficacy of the essential oils of *A. abyssinica* and *L. sativum* was assessed. The central analgesic effect of *A. abyssinica* and *L. sativum* essential oils was evaluated in mice using the hot plate method.
Results: Essential oil of *A. abyssinica* at all dose levels revealed a statistically significant ($p < 0.001$) analgesic activity in an acetic acid–induced peripheral pain model when compared to the negative control. The maximum analgesic activity (45.26%) was observed for the highest dose of *A. abyssinica* essential oil (400 mg/kg b.w.), which was comparable to aspirin (47.56%). The middle and lowest dose levels of essential oils of *A. abyssinica* resulted in 43.63% and 40.80% of inhibition, respectively. As compared to the vehicle-treated group, essential oil of *L. sativum* at all test dose levels showed a nonstatistically significant lowering of writhing in mice in a dose-dependent manner. In the hot plate method, when compared to the vehicle-treated group, the highest doses of both essential oils caused a highly significant ($p < 0.001$) delay in the reaction time at all observational intervals. At 30 min of observation, the standard drug's maximum analgesic activity (73.90%) was noted. At 90 min of monitoring, the maximum dose level of *L. sativum* essential was shown to have the most analgesic potential. Both plants exhibited similar central analgesic efficacy at this time point. At 120 min of observation, the highest dose level of *A. abyssinica* essential oil exhibited the maximum analgesic activity (51.25%); at equivalent doses, the effect was higher than *L. sativum* essential oil.
Conclusion: The traditional use of essential oils derived from *A. abyssinica* and *L. sativum* to treat headaches and other pain-related conditions is supported by this study.

Keywords: acetic acid; analgesic activities; *Artemisia abyssinica*; *Lepidium sativum*; mice

1. Introduction

Pain is an unpleasant, subjective, sensory, and emotional experience [1]. It impacts 80% of adult people globally [2] and is seen as the main clinical, social, and financial issue in the majority of communities [3]. Though uncomfortable, it serves as a warning signal and is essentially protective [4]. However, chronic conditions cause a significant decline in a person's health and quality of life and can have a negative impact on a patient's family [5]. Chronic pain, when untreated, is the most common condition that leads to physical injury and psychological issues [6].

The standard analgesics on the market have well-documented side effects that impact the hepatic system, cardiovascular system, renal system, and hematologic system [7]. Introducing selective cyclooxygenase-2 inhibitors has partially mitigated the gastrointestinal adverse effects of nonsteroidal anti-inflammatory medications. Nevertheless,

these substances were harmful to the heart muscles, glomeruli, hepatic cells, and cerebral cortex [8]. In addition to many adverse effects, which might include respiratory depression, sleepiness, decreased gastrointestinal motility, constipation, nausea and vomiting, pruritus, disruption of hormonal homeostasis, hearing loss, and tolerance, opioid analgesics have a high potential for addiction [9]. Given this, extensive research on medicinal plants that are supposedly useful in the treatment of pain is necessary [10].

Numerous traditional medicinal herbs are used by healers in various parts of Ethiopia to cure and manage pain. However, several of these medicinal plants' potential for therapeutic use has not yet been thoroughly examined and proven by science [10]. Plants with numerous medicinal uses in Ethiopian traditional medicine include *Artemisia abyssinica* and *Lepidium sativum*. These plants are used separately as a pain reliever by traditional medicine practitioners [11, 12].

A. abyssinica under the family of *Asteraceae* is known as "chikugn" in Ethiopia. It is a 30 –60 cm-tall, upright, annual, or transient perennial herb. It is often used in both rituals and conventional treatment. Many inflammatory illnesses, including rheumatism, headaches, fever, and diabetes, are treated with it in Ethiopia [13]. Additionally, reports of its use in the treatment of syphilis, leprosy, tonsillitis, gonorrhea, cough, and rabies have been made. Domestic animals with epilepsy can be treated with the fresh roots of the same herbs [14, 15]. As an anthelmintic, antispasmodic, antirheumatic, and antibacterial, the plant has also been utilized in traditional medicine. For the essential oil of *A. abyssinica*, there have also been reports of antioxidant, antileishmanial, and antitrypanosomal properties [13, 16]. The *A. abyssinica* oils from Ethiopia were rich in 4-hydroxycyclohexanemethanol (21.3%) and α-terpinolene (9.2%), yomogi alcohol (38.5%), artemisyl acetate (24.9%), and artemisia alcohol (6.7%) and 4, 5-dihydroxyocta-3, 5-diene-2, 7-dione (55.0%) [17].

Lepidium sativum is an edible herb belonging member of the Brassicaceae family. It is an annual plant, meaning it completes its life cycle in 1 year. The plant is known as "Garden cress" [18] in English. We refer to it as "fetto" in Ethiopia. It can be bought in every market and is frequently planted as a garden plant [19]. Garden cress has economic value in all of its parts, including its seeds, leaves, and roots [20]. But the plant is mostly grown for its seeds [21]. In traditional medicine, the leaves and seed oils are frequently used to treat a range of clinical difficulties, such as migraine, constipation, diarrhea, arthritis, fractures, hepatitis, asthma, hypertension, hyperglycemia, menstruation issues, and sexual debility [22–24]. Furthermore, pharmacological characteristics of its essential oils have been reported, including activity as a diuretic, immune system enhancer, antioxidant, laxative, febrifuge, and galactogogue. Natural antioxidants like tocopherols and carotenoids, as well as fatty acids like linolenic acid, are among the active compounds found in Lepidium sativum essential oils. Along with other substances like sinapic acid and sinapine, the oil may also contain alkaloids, including imidazole alkaloids and lepidine [25, 26].

Previous in vivo studies and available literature reported that *A. abyssinica* and *L. sativum* have analgesic properties [27–32]. However, published studies are not sufficient. The aim of the present study was to evaluate and compare the peripheral and central analgesic activities of essential oils of *A. abyssinica* and *L. sativum* in mice.

A research on the immunomodulatory effects of *A. abyssinica* and *L. sativum* essential oils on mice was published in January 2024. Both herbs may have analgesic properties since they strengthen immunity [33].

2. Materials and Methods

2.1. Chemicals, Reagents, and Drugs. Analytical grade materials, reagents, and chemicals were all used in this investigation. These include morphine and distilled water (EPHARM, Ethiopia), yeast extract powder (Foodchem International, China), acetylsalicylic acid (Bayer Schering Pharma AG, Germany), acetic acid (Shanghai Huayi, China), and 2% Tween 80 (Matangi, India).

2.2. Plant Materials. The seeds of *L. sativum* and the aerial components of *A. abyssinica* were gathered from Debre Berhan in North Shewa, Amhara National Regional State, Ethiopia, in September, 2022. Plants were verified at the biology department herbarium at Addis Ababa University in Ethiopia. The voucher specimens for *A. Abyssinica* and L. *sativum*, respectively, were placed in the herbarium with reference numbers AA/2022 and LS/2022.

2.3. Experimental Animals. For the experiments other than acute toxicity, Male Swiss albino mice, weighing 25–35 g and aged 2–3 months, were utilized. They were obtained from Ethiopian Public Health Institute in Addis Ababa, Ethiopia. Mice were housed in cages that had a 12 h light/dark cycle. They were fed regular pellets and given unlimited access to tap water. Five days before the trial, the animals were adjusted to the laboratory environment. The care and usage of the mice were done in accordance with the standards for experimental animals [34, 35].

2.4. Preparation of Essential Oils. Hydrodistillation was used to extract the essential oils of *A. abyssinica* and *L. sativum* from fresh seeds and aerial parts, respectively. The device used for extraction was a Clevenger type. For 8 hours, 1000 g of each fresh plant material was subjected to distillation. The yields obtained from essential oils of *A. abyssinica* and *L. sativum* were 2% and 20%, respectively. The extraction process was followed in accordance with the earlier methodology [36].

2.5. Acute Toxicity Study. The OECD 425 guideline's limit test recommendations were followed in the acute toxicity test for the essential oils of *A. abyssinica* aerial parts and *L. sativum* seeds conducted on female mice. Four hours before the experiment, three female Swiss albino mice were fasted. The essential oils were given to the mice orally via oral

gavage at a single dose of 2000 mg/kg body weight (b.w). Next, the mice were closely monitored for any physical or behavioral changes over the course of the 24 h, with extra attention paid to them during the first 4 hours. Four additional female mice received the same dosage of essential oils, and lethality was not noted during the first 24 h. For 4 weeks, careful observations were made on general toxicity signs and symptoms, food and water intake, and death [37]. The 4 week acute toxicity follow-up revealed that both essential oils did not cause any sign of physical, behavioral, and autonomic manifestations. Moreover, their feeding and water intake habits were not affected. No sign of death was also observed at the tested dose levels.

2.6. Dosing. In 2% Tween 80, essential oils were reconstituted [38]. From the acute toxicity result, the medina lethal dose of both essential oils was greater than 2000 mg/kg b.w and guideline recommendations were taken into consideration while choosing the dose levels [39].

2.7. Writhing Test. Eight groups of five mice each were formed. The vehicle was administered to group I orally and used as a negative control. As a positive control, group II was treated with 150 mg/kg b.w of acetylsalicylic acid orally [39]. *A. abyssinica* essential oil was given to groups III, IV, and V at doses of 100, 200, and 400 mg/kg b.w. p.o., respectively. *L. sativum* essential oil was administered to groups VI–VIII at doses of 100, 200, and 400 mg/kg b.w. p.o., respectively. Each group of mice received 0.35 mL of a 0.6% (v/v) acetic acid solution intraperitoneally (i.p.) after an hour of grouping. The number of writhes (abdominal constrictions) was counted for 20 min starting 10 min after the acetic acid was delivered. Inhibition against acetic acid-induced pain was indicated by a decrease in the mean number of pain responses using the following equation [40]:

$$\%\ \text{inhibition} = \frac{\text{Writhes in control} - \text{Writhes in test}}{\text{Writhes in control}} \times 100. \quad (1)$$

2.8. Hot Plate Method. The animal groups were similar to those used in the writhing test, with the exception of the positive control, which used morphine at a dose of 10 mg/kg b.w orally. The potential of the essential oils of *A. abyssinica* and *L. sativum* as central analgesics was assessed using this technique. The procedure involved inserting the mouse into an open-ended, cylindrical room with a metallic plate floor that was heated by a thermode. The behavioral components such as paw withdrawal and jumping were measured in terms of reaction times after a plate was heated to a constant temperature of 55°C ± 1°C. To prevent paw lesions, mice were individually placed on a hot plate with a 15 s cutoff period. The reaction time was measured as the delay before licking the paw or jumping off the hot plate. Following the delivery of the vehicle (10 mL/kg b.w p.o), the reference medication (10 mg/kg b.w orally) and 100, 200, and 400 mg/kg b.w orally of the essential oils, the reaction times were recorded at 0, 30, 60, 90, and 120 min. The following formula was used to determine the percentage increase in reaction time or pain threshold inhibition [10, 41]:

$$\%\ \text{elongation} = \frac{\text{latency (test)} - \text{latency (control)}}{\text{latency (test)}} * 100. \quad (2)$$

2.9. Data Reliability. To make sure that the outcomes could be repeated, each test was conducted twice. Since the data from the second experiment phase showed a high level of reliability with the first phase, it was considered for analysis.

2.10. Data Analysis. The data were analyzed using the statistical package for social science (SPSS) version 21 software and were displayed as mean ± SEM (standard error of mean). The statistical differences between the groups were compared using a one-way ANOVA, followed by a Tukey post hoc multiple comparison test. The results were considered significant when the p-value at a 95% confidence interval was less than 0.05.

3. Results

3.1. Acetic Acid-Induced Writhing Test. The peripheral analgesic activities of essential oils of *A. abyssinica and L. sativum* were determined in mice using acetic acid-induced writhing model. As indicated in Table 1 and Figure 1, the essential oil of *A. abyssinica* at all dose levels revealed a statistically significant ($p < 0.001$) analgesic activity in acetic acid-induced peripheral pain model when compared to the negative control. The maximum analgesic activity (45.26%) was observed for the highest dose of *A. abyssinica* essential oil (400 mg/kg b.w.), which was comparable to aspirin (47.56%). The middle and lowest dose levels of essential oils of *A. abyssinica* resulted in 43.63% and 40.80% of inhibition, respectively. As compared to the vehicle-treated group, the essential oil of *L. sativum* at the three test dose levels showed a nonstatistically significant lowering of writhing in mice in a dose-dependent manner. The effect was greatest at the highest dose level of the essential oil (400 mg/kg b.w.).

3.2. Hot Plate Method. The central analgesic effects of *L. sativum* and *A. abyssinica* essential oils were assessed in mice using this technique. The conventional drug morphine along with the essential oils of *A. abyssinica* and *L. sativum* at all dose levels demonstrated a considerable analgesic action in mice when compared to the negative control, as shown in Table 2. Comparing the positive treatment group to the vehicle-treated group, the former showed a substantial ($p < 0.001$) delay in reaction time at all-time intervals of observation. Similarly, mice's reaction times were considerably delayed when exposed to the essential oils of *A. abyssinica* and *L. sativum* in comparison to the negative control. The effect that was seen was dose-dependent. When compared to the vehicle-treated group, the highest doses of

Table 1: The effect of essential oils of *A. abyssinica* and *L. sativum* on acetic acid–induced writhing test in mice.

Group	Dose	Mean number of writhing (mean ± SEM)	% of inhibition (analgesic activity)
Vehicle	10 mL/kg	55.8100 ± 3.95433	
ASA	150 mg/kg	$29.2640 \pm 1.84801^{V***LS1***LS2**LS4**}$	47.56
AAEO1	100 mg/kg	$33.0380 \pm 1.81415^{V***LS1**LS2**}$	40.80
AAEO2	200 mg/kg	$31.4620 \pm 1.37107^{V***LS1**LS2**LS4*}$	43.63
AAEO4	400 mg/kg	$30.5520 \pm 1.66372^{V***LS1***LS2**LS4*}$	45.26
LSEO1	100 mg/kg	52.8100 ± 3.95433	5.37
LSEO2	200 mg/kg	49.8640 ± 3.95644	10.65
LSEO4	400 mg/kg	46.8280 ± 3.94688	16.09

Note: Results are expressed as mean ± SEM; $n = 5$. AAEO1: *A. abyssinica* essential oil at 100 mg/kg, AAEO2: *A. abyssinica* essential oil at 200 mg/kg, AAEO4: *A. abyssinica* essential oil at 400 mg/kg, LSEO1: *L. sativum* essential oil at 100 mg/kg, LSEO2: *L. sativum* essential oil at 200 mg/kg, LSEO4: *L. sativum* essential oil at 400 mg/kg, V = compared to the vehicle, LS1 = compared to L. *sativum* essential oil at 100 mg/kg, LS2 = compared to L. *sativum* essential oil at 200 mg/kg, and LS4 = compared to L. *sativum* essential oil at 400 mg/kg.
Abbreviation: SEM, standard error of the mean.
$^{*}p < 0.05$.
$^{**}p < 0.01$.
$^{***}p < 0.001$.

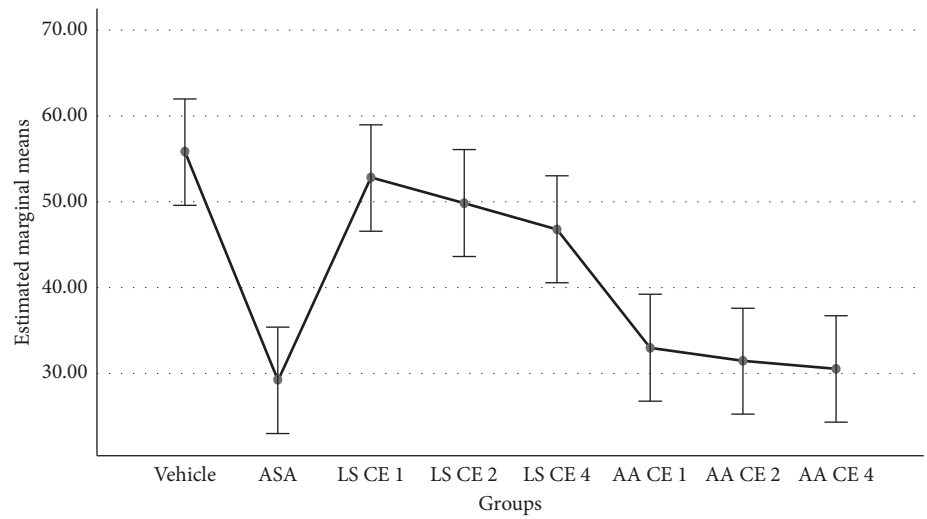


Figure 1: Estimated marginal means of essential oils of *A. abyssinica* and *L. sativum* on acetic acid–induced writhing test in mice. AACE1: *A. abyssinica* essential oil at 100 mg/kg, AACE2: *A. abyssinica* essential oil at 200 mg/kg, and AACE4: *A. abyssinica* essential oil at 400 mg/kg. LSCE1: *L. sativum* essential oil at 100 mg/kg, LSCE2: *L. sativum* essential oil at 200 mg/kg, and LSCE4: *L. sativum* essential oil at 400 mg/kg. ASA: acetyl salicylic acid. The result is expressed as mean ± standard error of the mean ($n = 5$). The error bars represent 95% CI of the mean generated during one-way ANOVA post hoc Tukey's test ($p \leq 0.05$). The true population mean will lay in between the upper and lower points of the 95% confidence interval in the error bar, and the smaller the CI, the higher precision of the sample mean for groups indicated on the horizontal axis. If the error bars overlap quite a bit in the range, there is no statistically significant difference between or among the average though there is a slight difference in the average response rate.

both essential oils caused a highly significant ($p < 0.001$) delay in reaction time at all observational intervals. At 30, 90, and 120 min of observation, mice administered with the middle dose of *A. abyssinica* essential oil demonstrated a significantly delayed reaction time than the negative control group ($p < 0.001$, $p < 0.5$, and $p < 0.01$, respectively). As compared to the negative control, mice treated with the middle dose of L. sativum essential oil showed a significant delay in reaction time at 30, and 120 min of observation ($p < 0.001$ and $p < 0.01$, respectively). At these time points, mice treated with the lowest dose of *A. abyssinica* essential oil show an increase in reaction time compared to the negative control group ($p < 0.01$ and $p < 0.001$, respectively). Only 30 min after treatment, mice given the minimal test dose of *L. sativum* essential showed a significant delay in reaction time when compared to the vehicle-treated group ($p < 0.001$).

Pretreatment values were similar across all groups, as Figure 2 illustrates. At 30 min of monitoring, the standard drug's maximum analgesic activity (73.90%) was noted (Table 3, and Figure 3). At 60 min of observation, the highest dose of *A. abyssinica* essential oil demonstrated a superior central analgesic action than *L. sativum* essential oil at comparable dose, as shown in Table 3 and Figure 4. At 90 min of monitoring, the highest dose level of *L. sativum* essential was shown to have the most analgesic potential.

Table 2: The effect of essential oils of *A. abyssinica* and *L. sativum* on the hot plate latency time (second) in mice.

Groups	Dose	Pretreatment value	30 min after treatment (mean ± SER)	60 min after treatment (mean ± SER)	90 min after treatment (mean ± SER)	120 min after treatment (mean ± SER)
Vehicle	10 mL/kg	1.910 ± 0.114	2.050 ± 0.039	2.370 ± 0.112	2.698 ± 0.180	2.726 ± 0.056
Morphine	10 mg/kg	2.080 ± 0.060	$7.854 \pm 0.098^{V***}$***LSEO1*****LSEO2*****LSE4*****AAEO1*****AAEO2*****AAEO4***	$8.626 \pm 0.170^{V***}$***LSEO1*****LSEO2*****LSEO4*****AAEO1*****AAEO2*****AAEO4***	$9.192 \pm 0.109^{V***}$***LSEO1*****LSEO2*****LSEO4*****AAEO1*****AAEO2*****AAEO4***	$8.508 \pm 0.160^{V***}$***LSEO1*****LSEO2*****LSEO4*****AAEO1*****AAEO2*****AAEO4***
LS EO	100 mg/kg	2.066 ± 0.057	$2.500 \pm 0.071^{V***}$	2.658 ± 0.031	2.986 ± 0.029	2.614 ± 0.104
	200 mg/kg	2.058 ± 0.056	$2.526 \pm 0.073^{V***}$***AAEO2*****AAEO4****	2.670 ± 0.034	3.062 ± 0.074	$3.534 \pm 0.172^{V***}$***LSEO1****
	400 mg/kg	1.954 ± 0.044	$2.760 \pm 0.010^{V***}$	$3.582 \pm 0.145^{V***}$***LSEO1*****LSEO2*****AAEO1*****AAEO2***	$4.536 \pm 0.150^{V***}$ ***LSEO1*****LSEO2*** AAEO1*****AAEO2***	$4.234 \pm 0.096^{V***}$***LSEO1*****LSEO2*
AA EO	100 mg/kg	2.060 ± 0.059	$2.494 \pm 0.052^{V***}$	2.728 ± 0.079	3.012 ± 0.040	$3.878 \pm 0.212^{V***}$***LSEO1***
	200 mg/kg	2.034 ± 0.046	$2.838 \pm 0.078^{V***}$ ***LSEO1****AAEO1*	2.784 ± 0.028	$3.310 \pm 0.106^{V*}$	$4.492 \pm 0.113^{V***}$***LSEO1*****LSEO2***
	400 mg/kg	1.974 ± 0.024	$2.910 \pm 0.048^{V***}$***LSEO1*****AAEO1***	$3.840 \pm 0.149^{V***}$ ***LSEO1*****LSEO2*** AAEO1*** AAEO2***	$4.634 \pm 0.125^{V***}$***LSEO1*****LSEO2*****AAEO1*****AAEO2***	$5.592 \pm 0.183^{V***}$***LSEO1*****LSEO2*****LSEO4*****AAEO1*****AAEO2***

Note: Results are expressed as mean ± SEM; $n = 5$. AAEO1: *A. abyssinica* essential oil at 100 mg/kg, AAEO2: *A. abyssinica* essential oil at 200 mg/kg, AAEO4: *A. abyssinica* essential oil at 400 mg/kg, LSEO1: *L. sativum* essential oil at 100 mg/kg, LSEO2: *L. sativum* essential oil at 200 mg/kg, LSEO4: *L. sativum* essential oil at 400 mg/kg, V = compared to the vehicle, LSEO1 = compared to L. *sativum* essential oil at 100 mg/kg, LSEO2 = compared to L. *sativum* essential oil at 200 mg/kg, and LSEO4 = compared to L. *sativum* essential oil at 400 mg/kg.

Abbreviation: SEM: standard error of the mean.

$^{*}p < 0.05$.

$^{**}p < 0.01$.

$^{***}p < 0.001$.

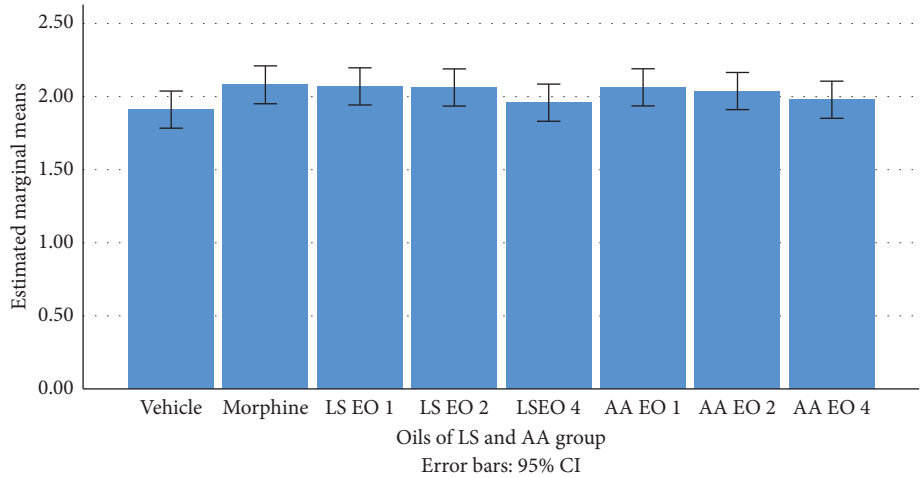


Figure 2: Estimated marginal means for pretreatment values of essential oils of *A. abyssinica* and *L. sativum* on thermal-induced pain in mice. AAEO1: *A. abyssinica* essential oil at 100 mg/kg, AAEO2: *A. abyssinica* essential oil at 200 mg/kg, and AAEO4: *A. abyssinica* essential oil at 400 mg/kg. LSEO1: *L. sativum* essential oil at 100 mg/kg, LSEO2: *L. sativum* essential oil at 200 mg/kg, and LSEO4: *L. sativum* essential oil at 400 mg/kg. The result is expressed as mean ± standard error of the mean ($n = 5$). The error bars represent 95% CI of the mean generated during one-way ANOVA post hoc Tukey's test ($p \leq 0.05$). The true population mean will lay in between the upper and lower points of the 95% confidence interval in the error bar, and the smaller the CI, the higher precision of the sample mean for groups indicated on the horizontal axis. If the error bars overlap quite a bit in the range, there is no statistically significant difference between or among the average though there is a slight difference in the average response rate.

Table 3: The effect of essential oils of *A. abyssinica* and *L. sativum* on the hot plate latency time (% increase in reaction time in mice).

Groups	Dose	% increase in reaction time			
		30 M	60 M	90 M	120 M
Vehicle	10 mL/kg				
Morphine	10 mg/kg	73.90	72.52	70.65	67.96
LSEO	100 mg/kg	18.00	10.84	9.64	−4.28
	200 mg/kg	18.84	11.24	11.89	22.86
	400 mg/kg	25.72	33.83	40.52	35.61
AAEO	100 mg/kg	17.80	13.12	10.42	29.71
	200 mg/kg	27.77	14.87	18.49	39.31
	400 mg/kg	29.55	38.28	41.78	51.25

Note: LSEO: *L. sativum* essential oil. AAE: *A. abyssinica* essential oil. M: minute after treatments.

Both plants exhibited a similar central analgesic efficacy at this time point (Figure 5). At 120 min of observation, the highest dose level of *A. abyssinica* essential oil exhibited the maximum analgesic activity (51.25%); at equivalent doses, the effect was higher than *L. sativum* essential oil (Figure 6).

4. Discussion

Finding effective antipain medications with few side effects makes sense, especially in light of the socioeconomic effects of pain [42, 43]. Finding medicinal plants with analgesic properties commonly utilized in the community to treat a variety of pain problems is crucial [10].

In Ethiopian folk medicine, *A. abyssinica* and *L. sativum* are often utilized for treating many conditions, including pain [13, 22–24]. There is not much in vivo scientific data to support the use of these plants for pain relief, despite conventional statements and some literature. Therefore, it could be valuable to perform a scientific research on the analgesic properties of the essential oils of *A. abyssinica* and *L. sativum* in mice.

The acetic acid-induced writhing test is used to determine the essential oil's peripheral analgesic activities. This test is used for reliable and rapid evaluation of analgesic actions. It is also sensitive and has the capacity to identify antinociceptive effects at dose levels, which remain inactive for other methods like tail flick [10, 44]. In this model, acetic acid administered intraperitoneally causes the release of prostaglandins and phlogistic mediators such as PGE2 and PGE2a. Visceral pain is thought to be modeled by the acetic acid-induced writhing test. Through the activation of primary afferent nociceptors and an increase in capillary permeability, prostaglandin elevation in the peritoneal cavity intensifies inflammatory pain. The analgesic receptors' sensitization to prostaglandins is linked to the constrictions that occur in the abdomen following the administration of acetic acid [29, 45, 46].

In the current study, essential oil of *A. abyssinica* at all dose levels revealed a statistically significant ($p < 0.001$) analgesic activity in acetic acid-induced peripheral pain model in mice when compared to the negative control group. Regarding the % of inhibition of writhing, the maximum analgesic activity (45.26%) was observed for the maximum dose of the essential oil of *A. abyssinica* (400 mg/kg b.w.), which was comparable to aspirin (47.56%). The middle and lowest dose levels of *A. abyssinica* essential oil resulted in 43.63% and 40.80% of inhibition, respectively. Essential oil of *L. sativum* at the three test dose levels showed a nonstatistically significant lowering of writhing in mice in a dose-dependent manner. Similarly, the effect was greatest at the highest dose level (400 mg/kg). When the peripheral analgesic was compared between the two plants,

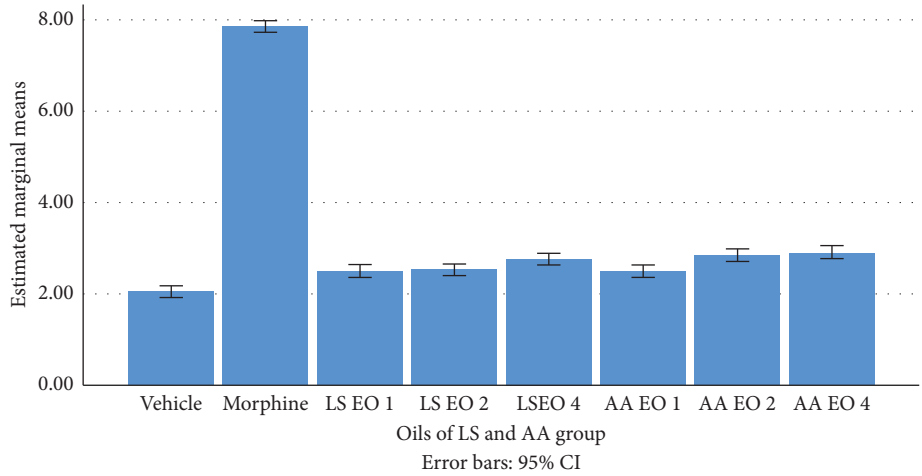


Figure 3: Estimated marginal means of the effects of essential oils of *A. abyssinica* and L. *sativum* on thermal-induced pain in mice, 30 min after treatment. AAEO1: *A. abyssinica* essential oil at 100 mg/kg, AAEO2: *A. abyssinica* essential oil at 200 mg/kg, and AAEO4: *A. abyssinica* essential oil at 400 mg/kg. LSEO1: *L. sativum* essential oil at 100 mg/kg, LSEO2: *L. sativum* essential oil at 200 mg/kg, and LSEO4: *L. sativum* essential oil at 400 mg/kg. The result is expressed as mean ± standard error of the mean ($n = 5$). The error bars represent 95% CI of the mean generated during one-way ANOVA post hoc Tukey's test ($p \leq 0.05$). The true population mean will lay in between the upper and lower points of the 95% confidence interval in the error bar, and the smaller the CI, the higher precision of the sample mean for groups indicated on the horizontal axis. If the error bars overlap quite a bit in the range, there is no statistically significant difference between or among the average though there is a slight difference in the average response rate.

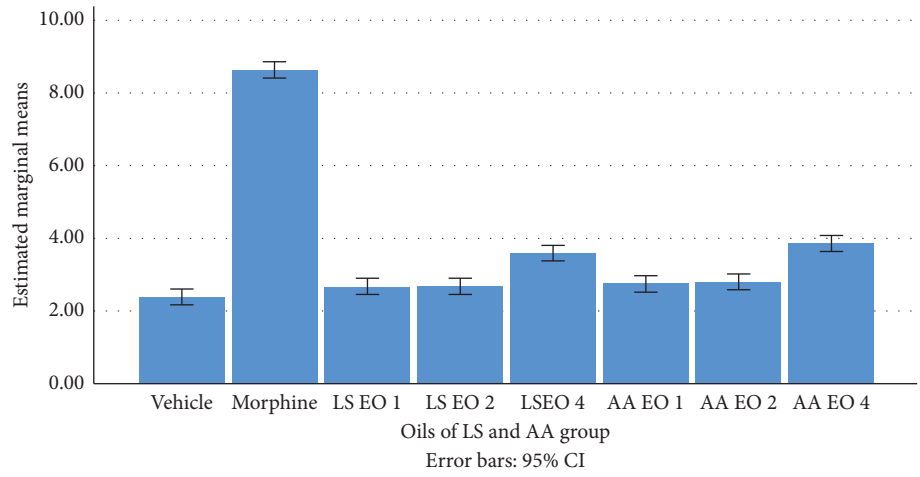


Figure 4: Estimated marginal means of the effects of essential oils of *A. abyssinica* and L. *sativum* on thermal-induced pain in mice, 60 min after treatment. AAEO1: *A. abyssinica* essential oil at 100 mg/kg, AACE2: *A. abyssinica* essential oil at 200 mg/kg, and AAEO4: *A. abyssinica* essential oil at 400 mg/kg. LSEO1: *L. sativum* essential oil at 100 mg/kg, LSEO2: *L. sativum* essential oil at 200 mg/kg, and LSEO4: *L. sativum* essential oil at 400 mg/kg. The result is expressed as mean ± standard error of the mean ($n = 5$). The error bars represent 95% CI of the mean generated during one-way ANOVA post hoc Tukey's test ($p \leq 0.05$). The true population mean will lay in between the upper and lower points of the 95% confidence interval in the error bar, and the smaller the CI, the higher precision of the sample mean for groups indicated on the horizontal axis. If the error bars overlap quite a bit in the range, there is no statistically significant difference between or among the average though there is a slight difference in the average response rate.

A. abyssinica demonstrated greater response at all comparable doses. This suggests that the essential oil of *A. abyssinica* has a higher concentration of secondary metabolites with analgesic properties. Likewise, an increase in the concentration of phytoconstituents that exhibit analgesic activity at the highest dose may be responsible for the analgesic activity that increases with increasing doses of the essential oils. Our findings imply that these essential oils' pharmacological mechanism for their analgesic impact may be partially associated with a decrease in prostaglandin synthesis due to their inhibitory role in the lipooxygenase and/or cyclooxygenase pathway [45, 47]. The impact of L. sativum essential oil on writhing caused by acetic acid is consistent with findings from another investigation [6].

The other model employed was the hot plate test, which revealed the participation of the central antinociceptive system as well as supraspinal nociception. The central antinociceptive properties of the essential oils were

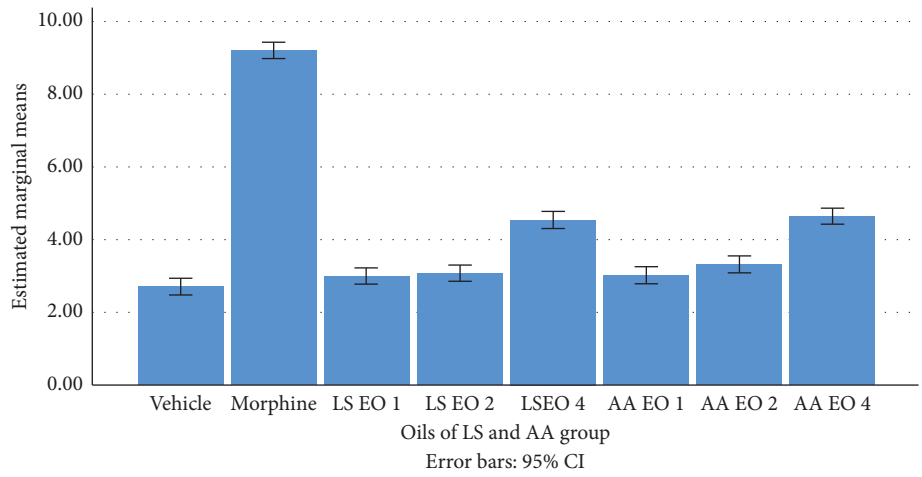


Figure 5: Estimated marginal means of the effects of essential oils of *A. abyssinica* and L. *sativum* on thermal-induced pain in mice, 90 min after treatment. AAEO1: *A. abyssinica* essential oil at 100 mg/kg, AAEO2: *A. abyssinica* essential oil at 200 mg/kg, and AAEO4: *A. abyssinica* essential oil at 400 mg/kg. LSEO1: *L. sativum* essential oil at 100 mg/kg, LSEO2: *L. sativum* essential oil at 200 mg/kg, and LSEO4: *L. sativum* essential oil at 400 mg/kg. The result is expressed as mean ± standard error of the mean ($n = 5$). The error bars represent 95% CI of the mean generated during one-way ANOVA post hoc Tukey's test ($p \leq 0.05$). The true population mean will lay in between the upper and lower points of the 95% confidence interval in the error bar, and the smaller the CI, the higher precision of the sample mean for groups indicated on the horizontal axis. If the error bars overlap quite a bit in the range, there is no statistically significant difference between or among the average though there is a slight difference in the average response rate.

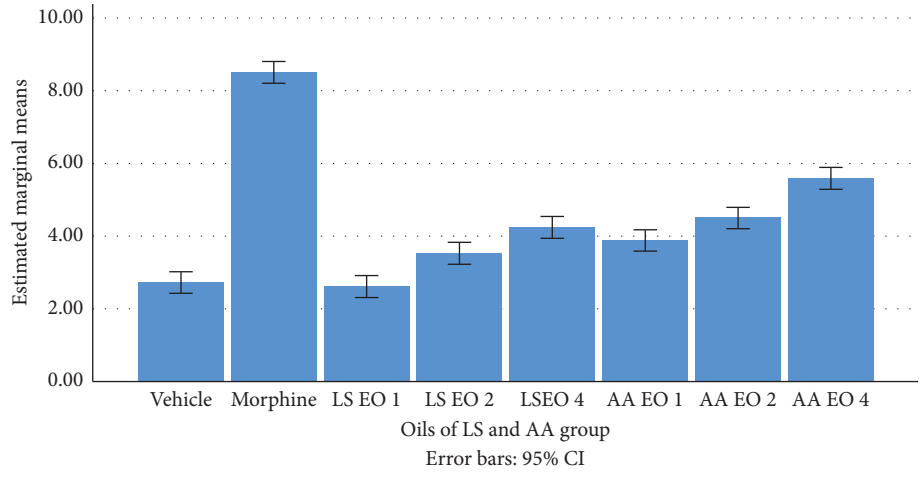


Figure 6: Estimated marginal means of the effects of essential oils of *A. abyssinica* and L. *sativum* on thermal-induced pain in mice, 120 min after treatment. AAEO1: *A. abyssinica* essential oil at 100 mg/kg, AAEO2: *A. abyssinica* essential oil at 200 mg/kg, and AAEO4: *A. abyssinica* essential oil at 400 mg/kg. LSEO1: *L. sativum* essential oil at 100 mg/kg, LSEO2: *L. sativum* essential oil at 200 mg/kg, and LSEO4: *L. sativum* essential oil at 400 mg/kg. The result is expressed as mean ± standard error of the mean ($n = 5$). The error bars represent 95% CI of the mean generated during one-way ANOVA post hoc Tukey's test ($p \leq 0.05$). The true population mean will lay in between the upper and lower points of the 95% confidence interval in the error bar, and the smaller the CI, the higher precision of the sample mean for groups indicated on the horizontal axis. If the error bars overlap quite a bit in the range, there is no statistically significant difference between or among the average though there is a slight difference in the average response rate.

identified by exposing the mice to the continuously heated plate and monitoring their reaction times, which included jumping, withdrawing, and licking of the paws. This model was chosen due to its sensitivity to potent analgesics and its limited tissue damage with a 15 s cutoff time, which is often used to restrict the mouse's exposure to the hot plate. Additionally, the model saves time and typically yields accurate readings [48].

In comparison to the negative control, mice treated with *A. abyssinica* and *L. sativum* essential oils at all dose levels and the standard medication morphine demonstrated a significant central analgesic effect. Comparing the positive treatment group to the vehicle-treated group, the former showed a substantial ($p < 0.001$) delay in reaction time at all-time intervals of observation. Mice's reaction time delays were observed when exposed to the essential oils of

A. abyssinica and L. sativum in comparison to the negative control. The effect seen was dose-dependent. When compared to the vehicle-treated group, the highest doses of both essential oils caused a highly significant ($p < 0.001$) delay in reaction time at all observational intervals. The existence of a high concentration of active metabolites may account for the comparatively greater activity of 400 mg/kg of these essential oils. Time dependence was also shown for the analgesic activity. When the essential oils of both plants were first observed, there was a noticeable increase in reaction time, which could mean that the analgesic impact started quickly. After 30 min of monitoring, the greatest analgesic activity (73.90%) for the reference medication was noted. The maximum concentration of *A. abyssinica* essential oil demonstrated better central analgesic efficacy during 60 min of observation compared to *L. sativum* essential oil at comparable dose. After 90 min of monitoring, the highest dose level of *L. sativum* essential was shown to have the most analgesic potential. Both herbs exhibited a similar central analgesic effect at this time point. At 120 min of observation, the maximum dose level of *A. abyssinica* essential oil showed the greatest analgesic action (51.25%); at a comparable dose, the impact was stronger than that of *L. sativum* essential oil. This indicated that *A. abyssinica* essential oil acts for a longer period of time. Essential oil of *A. abyssinica* may take longer to enter the central compartment and distribute to the target site, or for active metabolites to form that have a longer half-life and capable of producing analgesic effects [10].

One explanation for the essential oils' potential central analgesic effects could be the activation of the periaqueductal gray matter (PAG), which releases endogenous peptides like enkephalin and endorphin. These endogenous peptides descend the spinal cord and act as peripheral mechanisms that inhibit prostaglandins, leukotriene, and other endogenous substances, which are important players in central pain transmission, or act as inhibitors of the pain impulse transmission at the synapse in the dorsal horn [49].

The phytoconstituents found in these plants may be responsible for their analgesic properties. Studies revealed that the essential oils of *A. abyssinica* and *L. sativum* contained both flavonoids and alkaloids [18, 50, 51]. The synthesis of prostaglandins is inhibited by flavonoids [49]. Alkaloids produce analgesic effects by interfering with neurotransmitters that increase pain perception in the central nervous system [52]. The A. abyssinica oils from Ethiopia were rich in 4- hydroxycyclohexanemethanol (21.3%) and α-terpinolene (9.2%), yomogi alcohol (38.5%), artemisyl acetate (24.9%), and artemisia alcohol (6.7%), and 4, 5-dihydroxyocta-3, 5-diene-2, 7-dione (55.0%). These secondary metabolites might be responsible for the analgesic activities of the plant oil [17]. L. *sativum* essential oil may also contain alkaloids, including imidazole alkaloids and lepidine, which might have a positive implication in central and peripheral analgesic effects [25, 26].

Essential oils isolated from *A. abyssinica* and *L. sativum* demonstrated significant analgesic effects in both hot plate and acetic acid-induced writhing tests.

5. Conclusion

It can be concluded that essential oils of *L. sativum* and *A. abyssinica* have analgesic properties. Our results show that the essential oil of *A. abyssinica* has a greater analgesic effect than that of *L. sativum* essential oils. Future studies on the molecular mechanisms of action, thorough toxicity profiles, and phytochemical characterization of these essential oils can be planned.

Nomenclature

ANOVA	Analysis of variance
ASA	Acetylsalicylic acid
CI	Confidence interval
IP	Intraperitoneal
LD50	Median lethal dose
M	Minute
OECD	Organization for Economic Cooperation and Development
P.O	Per Os (by mouth)
SPSS	Statistical Package for Social Science

Data Availability Statement

The findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The Institutional Review Board of Debre Berhan University in Ethiopia approved the study before its start. A clearance letter was given. Furthermore, every safety measure was taken to ensure that the animals in the trial did not suffer. Every technique was used in compliance with all applicable rules and regulations. Reports on all methodologies took ARRIVE guidelines into consideration.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Each author contributed significantly to the work reported, whether it was through ideation, study design, execution, data acquisition, analysis, and interpretation, or in all of these areas. They approved the final version submitted. All agreed to take responsibility for the work in its entirety.

Funding

We are very grateful to Debre Berhan University for funding.

Acknowledgments

We are very grateful to Debre Berhan University for funding and Addis Ababa University for providing laboratory facilities. We are thankful to Ethiopia's Ethiopian Public Health Institute for lending us the experimental mice.

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