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Original Research Article

Pain Alleviating Potential of Ethanol Extract of *Vernonia amygdalina* Del. on Writhing in Male Wistar Rats

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ABSTRACT

Non-steroidal anti-inflammatory drugs and central analgesics are both effective pain relievers, but they have limitations and possible adverse effects. In African, Indian, and Chinese folkloric medicine, *Vernonia amygdalina* (VA) is known widely for its pharmacological benefits including antioxidant, antibacterial, and anti-cancer properties. The models, acetic acid-induced writhing, hot-plate latency, and tail-flick response, were utilized in this study for assessing the anti-inflammatory and analgesic potential of VA, alongside its toxicological study. The toxicology results showed no sign of toxicity, including mortality, salivation, diarrhea, or abnormal weight loss or gain. The serum AST and ALT were within the reference range (ALT:7-45 U/l; AST: 6-38 U/l). The analgesic effect of VA extract was highly demonstrated after 3 days of pretreatment at the two highest doses (400 and 800 mg/kg) in a dose-dependent manner. Despite being less effective than the larger doses, an analgesic effect was also observed at 200 mg/kg, which implies that VA may still have some analgesic effects at lower doses. The higher doses of VA were as effective and provided a prolonged analgesic effect that was comparable to the conventional medications (Diclofenac and Aspirin) used. The analgesic effect of VA has been suggested to be associated with its rich alkaloid constituent, which has been reported to exert an analgesic effect via mechanisms such as opioid receptor activation, neurotransmitter modulation, inhibition of pro-inflammatory cytokines, and ion channel modulation, among others. Hence, this study supports the utilization of VA as alternative therapeutic approach for the alleviation of pain and its symptoms.

***Keywords*:** Pain, Analgesic, Anti-inflammatory, Acetic Acid-induced, Traditional Medicine.

Introduction

Pain, as described by the International Association for the Study of Pain (IASP), can be referred to as an experience of sensory and emotional distress that is linked with a potential or real tissue damage.[1,2] Pain can be localized, as in an injury, or it can be diffuse, as in illnesses such as fibromyalgia. According to Craig and MacKenzie,[3] pain is a complex experience with sensory, physiological, cognitive, emotional, and behavioral components. By altering the way noxious stimuli are transmitted to the brain, emotions, behavioral responses to pain, beliefs, and attitudes influence the way pain is felt. Pain is classified as nociceptive, which is an acute reaction to a mechanical insult or noxious stimuli; inflammatory, which is related to damage to the tissue and immune cell intrusion; and pathological (neuropathic pain) as a result of nervous system damage.[4] Pain may result in a decreased quality of life and an increased health expenditure.[5]

Analgesics is any member of the group of medications that reduce or completely eliminate the pain associated with many pathologic conditions.[6,7] The mechanism of their actions is by interrupting signals caused by pain, from reaching the brain or by impeding the way the brain interprets the signals it receives.[8,9]

Analgesics may predominantly act on the central nervous system – opioids, which include morphine, codeine, heroin etc., or peripheral nervous system – non-opioids such as COX-2 inhibitors, acetaminophen, and nonsteroidal anti-inflammatory drugs (NSAIDs).[10] While central analgesics and NSAIDS are both potent pain relievers, they have limitations and potential side effects. NSAIDs have the potential to irritate the stomach lining, which can result in gastritis, ulcers, or bleeding.[11,12,13] They also raise the chance of having a heart attack or stroke, having impaired renal function, having allergic reactions, and interfering with blood clotting.[14,15] Central analgesics can cause sleepiness, disorientation, and reduced concentration, impairing focus and coordination.[16] Tolerance and dependence may be developed after extended usage, resulting in withdrawal symptoms. Opioid-based central analgesics frequently cause constipation, and at high dosages, they can depress the respiratory center and cause fatal consequences.[17,18,19] Hence, there is a need to explore medicinal plants as an alternative therapy for more effective and safer analgesics.

Many plant-derived products, also known as herbal medicine, are commonly utilized by Chinese, African, and Indian traditional healers for treating, preventing, and managing numerous diseases, many of which have been reported for their analgesic properties.[20,21] Although there is a need for more effective and safer analgesics for long-term use and medicinal plants present viable alternatives with fewer negative effects owing to their abundance of natural chemicals, using natural therapies also comes with some dangers and potential drug interactions. *Vernonia amygdalina* Delile, also referred to as the South African leaf, is a plant with tropical African origins.[22] The woody shrub belonging to Asteraceae family commonly found in humid tropical forests of Sub-Saharan Africa, Southeast Asia, and the southern coastal provinces of China such as Guangdong, Fujian, and Hainan.[22,23] To improve body health and immunity, *V. amygdalina* (VA) dry leaves undergo

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processing into a natural healthy product (herbal tea) in the southeast coast region of China.[24] Boiling or soaking the dark green leaves in fresh water might lessen their bitter taste.[25] The leaves are used in Nigeria to make the well-known bitter leaf soup, and the stem and root are utilized as chewing sticks.[26]

VA leaves are used for folk medicine and have been found to contain bioactive phytochemicals such as alkaloids, flavonoids, phenolic acid, xanthones, terpenes, coumarins, lignan, saponin, sesquiterpene lactones, epivernodalol, elemanolide, edotides, anthraquinones, steroids, coumarins, and anthraquinone.[22,27,28,29] VA has been discovered in numerous investigations to have therapeutic potential due to its antidiabetic, anthelmintic, antimalarial, antioxidant, antimicrobial, and anti-cancer properties.[28,30] Traditional medicines are widely utilized for treatment across the globe because of their therapeutic potentials that are comparable to those of conventional pharmaceuticals, their availability, accessibility, cost-effectiveness, and zero or minimal adverse effects when administered in a safe dosage or concentration.[31] Although previous studies may have explored the general analgesic properties of VA, a knowledge gap still exists on the ethanol extract from the study plant and its impact, specifically on writhing behavior and associated symptoms. Therefore, this study seeks to determine a safe dosage for the ethanol extract of VA and to investigate its analgesic, as well as its anti-inflammatory effects on writhing induced via acetic acid, in male Wistar rats and its potential mechanism of action.

Materials and Methods

Chemicals and drugs

Ethanol, 0.6% Acetic Acid, Acetylsalicyclic Acid (Aspirin), Diclofenac, Normal Saline, and Distilled Water. Other reagents and chemicals utilized were of commercial analytical grade.

Plant collection and identification

The leaves of VA were harvested at Babcock University, Ilishan-Remo, Ogun State premises, in December 2022. The plant was initially identified by one of the researchers, Dr. Aderiike Adewumi, a plant taxonomist and systematist in the Department of Basic Sciences and further authenticated by Prof. Cyril Nwangburuka, a Professor of Plant Breeding in the Department of Agriculture and Industrial Technology. A voucher specimen (BU/SAT/22-091) was deposited at the herbarium section of the department.

Animal Purchase and Handling

Seven-week-old male Wistar rats of weight ranging between 150 and 170 g were procured from Ladoke Akintola University of Technology (LAUTECH), in the animal care unit of the Department of Animal Production and Health. All experimental animals were housed and handled following the approved guideline for the use and care of laboratory animals of the US National Institutes of Health (NIH). Animals were housed at monitored room temperature (25 ℃ ± 2 ℃) with a 12-h light/dark cycle *ad libitum* to standard pellet feed and water, in poly-propylene cages for 14 days for the purpose of acclimatization. An ethical approval (approval no. BUHREC 1022B/23) for the use of experimental animals was obtained from the Babcock University Research Ethics Committee (BUHREC).

Plant Extraction

The harvested leaves of VA were gently washed, after which the leaves were air dried under shade, and blended into powder. The ethanol extraction of the sample was according to the Owoyele *et al*.[32] method, with slight modifications. Three hundred grams (300 g) of blended leaves were macerated in 80% ethanol (1:10) for 3 days (72 h) at room temperature. The extract was separated after maceration from the residue with an assembly filtration and evaporated at 60 ℃ in a water bath and later at a lowered temperature (40 ℃) using a rotatory evaporator. The gel-like semi-solid dark green extract weighed 41.39 g (13.8% of the total sample) and was stored at 4 ℃ for subsequent analysis.

Acute Toxicity

Male Wistar rats of weight ranging between 180 and 200 g were grouped into four groups (n = 5). Group I received normal saline and represents the control group; Group II, III, and IV received a single-dose oral administration of an ethanol extract of VA dissolved in normal saline at 200, 400, and 800 mg/kg, respectively. The experimental rats were weighed before the study and at intervals throughout the study. Other behavioral changes, including mortality, weight change, diarrhea, and others, were observed and recorded at 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h subsequent intervals for 3 days. The study animals were fasted overnight, euthanized, and blood samples were collected for liver function assays (AST and ALT).

Acetic Acid-Induced Writhing Response

The procedure of Koster *et al*.[33] as explained earlier by Shehu *et al*.[34] was employed to assess the acetic acid-induced writhing in the experimental rats. Male Wistar rats (average weight = 189.2 g) were grouped into five (n = 5) and pretreated with oral administration of normal saline (Group I – normal control); 40 mg/kg Aspirin (Group II); and 200, 400, and 800 mg/kg VA (Groups III, IV, and V, respectively) for 3 days. The rats, at 1 h after administration of the final pretreatment, were injected with 0.6% v/v of acetic acid solution in water (10 ml/kg i.p.) for pain induction. The writhing number over the period of 0–30 min (cut-off time) was observed and recorded.

Hot Plate Latency

This test was done using the approach of Eddy and Leimbach,[35] where experimental rats were grouped into five (n = 5). Group I was pretreated with saline (control); Group II was pretreated with 25 mg/kg Diclofenac; and Groups III, IV, and V were earlier treated with 200, 400, and 800 mg/kg ethanol extract of VA, respectively. The pretreated rats were placed individually in the glass compartment of an Eddy hot plate set to 55 ± 0.5 ℃. The index of response latency was measured as the duration of time required for either jumping or paw-licking. The latency response was measured and recorded at 0, 30, 60, 120, and 180 min. A cut-off time of 30 s was set to avoid burn risk.

Tail-Flick Test

This evaluation was done using the method of Hayashi and Takemori.[36] The experimental rats were also administered treatment (normal saline in Group I; 25 mg/kg Diclofenac in Group II; 200, 400, and 800 mg/kg ethanol extract of VA in Groups III, IV, and V, respectively) prior to the tail-flick response assessment. The tail tip of pretreated rats was then immersed in hot water at 50.0 ± 1.0 ℃, while thermal responses were monitored and recorded at 0, 30, 60, 120, and 180 min.

Data Analysis

Results were expressed as mean ± S.E.M. in each group. Differences between groups for different parameters obtained during the study were evaluated by One-Way ANOVA and Post hoc Tukey test. A value of $p < 0.05$ was considered a significance level.

Results and Discussion

VA is one of the most common plants in Africa and Asia, with nutritional and medicinal values. The pharmacological effects, such as antioxidant,[37] antipyretic,[38] anti-cancer,[39] anti-malaria,[40] anti-diabetic,[30] among others, are a result of its rich phytoconstituents, including flavonoids, alkaloids, triterpenoids, saponins, steroids, tannins, etc.[31,39,41] This study evaluates the toxicity level of this plant because of its extensive utilization as food and medicine to ensure its safe dosage for our subsequent experiment. The toxicity signs considered include mortality, salivation, diarrhea, changes in feces color and weight, and any other irregular behavioral pattern.[42,43,44] The results in Table 1 show the toxic effect of the ethanol extract of VA on the liver function markers, which indicates that there was no damage caused to the liver after assessing the ALT and AST levels. When comparing the average AST concentration (23.66±0.98) at 800 mg/kg VA treatment to the control group and other lower dosages 200 and 400 mg/kg, there was a significant ($p < 0.01$) difference. Additionally, there was no discernible difference between the 200 and 400 mg/kg VA administration groups

and the control group. Likewise, an analysis of the average ALT concentration at 800 mg/kg (20.15 ± 1.09) revealed a significant difference $p < 0.01$ in comparison to the control group (17.56 ± 0.45), but a significant difference $p < 0.05$ after 400 mg/kg VA treatment (18.77 ± 0.62). However, no significant difference was observed in the 200 mg/kg VA administration group. Since the liver function test reference range varies based on the laboratory in which the test was carried out, it is recommended that each laboratory establish its own reference interval based on its methodology.[45] Although there was a slight significant difference in the concentration of the liver markers, there was no sign of damage since they fall within the laboratory reference range (ALT: 7–45 U/l; AST: 6-38 U/l) for the liver function assay.

Furthermore, there was a steady pattern in the weight increments of the study animals. Table 2 represents the result for the effect of the plant extract on the weight of the experimental animals. While toxic chemicals can influence body weight either through gain or loss of weight, the results showed no significant difference in the percentage weight gain at 200 and 800 mg/kg (1.66% and 1.63%, respectively) when comparing with the control group (1.76%). Meanwhile, a significant ($p < 0.05$) difference was observed in the percentage weight gain at the 400 mg/kg extract treatment (2.00%). However, the significant percentage weight gain observed at 400 mg/kg may not be an indication of a toxic effect since the observation was not in a dose-dependent manner. Some other biological variations, such as the amount of feed intake, metabolism, and genetic factors, may have an influence on the weight of the individual.[46,47,48] Additionally, physical changes, including mortality, salivation, diarrhea, changes in feces color, and other abnormal behavioral responses, were observed to ascertain the toxicity effect of the plant extract. There were zero deaths recorded among all the experimental treatment groups, no sign of physical changes, or any abnormal behavioral response. Similarly, the behavioral changes assessed in this study were the considered sign of toxicity in a previous study reported by Yaghoubi *et al*.[49] The study findings corroborate with our finding that zero mortality, as well as no behavioral changes, after the administration of VA at 200, 400, and 800 mg/kg, is an indication of no toxicity.

Analgesics are medications that selectively reduce pain without significantly affecting consciousness by acting on the peripheral or central nervous system.[49,50] Centrally-acting analgesics work by lowering the pain threshold and changing the body's physiological reaction to pain.[51,52,53] In contrast, the production of impulses at the chemoreceptor site of pain is inhibited by peripherally acting analgesics.[51,54,55,56] Thermal stimuli (hot plate latency and tail-flick test) and acetic acid-induced writhing were the pain-state model used in this study to screen for analgesic effectiveness of ethanol extract of VA in animal models. The hot plate latency and tail-flick test approach are thought to be a supraspinal-structured reaction that includes higher brain functions.

Using acetic acid-induced writhing as a pain model, the experiment was designed to evaluate the peripheral analgesic potential of VA in Wistar rats. The amount of writhing or abdominal contractions was counted after giving the rats various dosages of ethanol extracts of VA. It was obvious, as shown in Table 3, that the ethanol extracts of VA had a considerable analgesic effect as compared to the normal control group. The 400 mg/kg and 800 mg/kg, which were the two highest doses, showed the most significant ($p < 0.01$) decreased number of writhing in a dose-dependent manner. This dose-dependent pattern indicates that VA may possess an analgesic quality, and this analgesic effect is dependent on the amount of dose, i.e., it became more evident as the dosage was increased. This finding correlates with the general perception about analgesics that the drugs exhibit a dose-dependent response, with higher dosages often resulting in a more pain-relieving effect.[57,58] Furthermore, the outcomes showed a significant ($p < 0.01$) reduced number of writhing even at a lower dose of 200 mg/kg when compared to the saline control. Although it was less effective than the higher doses, this implies that VA may still have some analgesic effects at lower doses.

Additionally, VA dosages of 200 and 400 mg/kg demonstrated a considerably distinct analgesic effect when compared to aspirin. This implies that VA may be effective in relieving pain in this animal model when used at these doses, but may not be as effective as conventional medication. However, no significant difference was found between the 800 mg/kg dose of VA and the aspirin treatment, indicating that at this high dose, VA's analgesic impact may become equivalent to that of aspirin. The study findings support an earlier studies that revealed the analgesic effects of various medicinal plants with dose-dependent activity and greater effectiveness at higher dosages, comparable to conventional medications.[59,60,61]

To assess the analgesic effect of the ethanol extract of VA, a hot-plate latency method was used. The results presented in Table 4 offer a valuable insight into the analgesic potential of the studied plant and standard drug over several duration intervals while comparing the treatment groups against the control group. Initially, there were no significant differences observed among all treatment groups at the 0 min start point. This implies that all groups have a relatively equal baseline for pain response at the start of the experiment. This serves as a reference point for measuring and comparing subsequent assessments.

However, the study found that the 400 and 800 mg/kg ethanol extract treatment groups of VA showed significant ($p < 0.001$) analgesic effects compared to the control group between a 30- and 180-min time range (30-min interval), indicating their potential as an alternative analgesic agent. This finding suggests that both doses of VA were effective in reducing pain perception, which aligns with the concept of dose-dependent analgesia and demonstrated the potential analgesic properties of VA.[57] The 800 mg/kg dose showed no significant difference between the VA and Diclofenac treatment groups. At the 400 mg/kg dose, there were significant differences at 60 and 180 min, but no significant difference at other time points. The 200 mg/kg dose of VA showed a significant difference, especially at 180 min, suggesting that the lower dose might not be as effective as Diclofenac in providing analgesia.

Table 1: Effect of Ethanol Extract of ***VA*** on Serum Marker Enzymes for Liver Function

Treatment Group	Administration	AST (U/L)	ALT (U/L)
Group I	Control (Saline)	21.31 ± 1.13	17.56 ± 0.45
Group II	VA (200 mg/kg)	20.63 ± 0.17	17.38 ± 0.32
Group III	VA (400 mg/kg)	22.04 ± 1.45	18.77 ± 0.62[a]
Group IV	VA (800 mg/kg)	23.66± 0.98[b]	20.15 ± 1.09[b]

Values are expressed as mean ± standard error of mean (n = 5). a – means significant difference at p<0.05, when compared with control; b – means significant difference at p<0.01, when compared with control

Table 2: Effect of Ethanol Extract of *VA* on the weight of experimental animals

Treatment Group	Administration	Average Initial Weight (g)	Average Final Weight (g)	Percentage Gain (%)
Group I	Control (Saline)	188.76 ± 0.45	192.15 ± 0.36	1.76
Group II	VA (200 mg/kg)	196.45 ± 1.22	199.72 ± 1.15	1.66
Group III	VA (400 mg/kg)	193.32 ± 0.65	197.26 ± 0.72	2.00[a]
Group IV	VA (800 mg/kg)	191.18 ± 0.33	194.34 ± 1.28	1.63

Values are expressed as mean ± standard error of mean (n = 5). a – means significant difference at p<0.05

These findings align with previous studies, which provide an understanding that the effectiveness of analgesic agents can vary depending on the dose administered and the duration of action.[62,63] The higher doses of VA were as effective as the standard drug, promising further investigation. However, the safety and long-term effects of VA as an analgesic should also be explored in future research to fully understand its potential clinical utility.

Similar to the observations in the hot-plate latency method, the result presented in Table 5 shows that, no observed significant difference at 0 min among all the treatment groups, and this also served as a baseline pain response reference for subsequent comparison between the treatment and the control group. Meanwhile, notable and significant differences were observed between 30 and 180 min, which suggests a substantial analgesic potential of VA when compared to the control group within the same time interval. There was a significant ($p<0.01$) difference at 30 min between the Diclofenac, 400, and 800 mg/kg extract treatment groups, while a significant ($p<0.05$) difference was observed at the 200 mg/kg extract treatment group.

However, the analgesic effect became more distinct, with a significant difference ($p<0.001$) observed at 60 to 120 min in the Diclofenac, 400, and 800 mg/kg extract treatment groups. A significant difference ($p<0.05$) was also observed in the 200 mg/kg treatment groups for the same time range. This implies that higher VA doses provided a prolonged analgesic effect that was comparable to the conventional medication, diclofenac.

Furthermore, there was no significant difference observed at the 800 mg/kg extract treatment group when compared with the Diclofenac treatment groups, but at the 200 and 400 mg/kg extract treatment groups, there was a significant difference observed, $p<0.001$ at 60 to 120 min and $p<0.01$ at 180 min of the 200 mg/kg extract treatment group. A significant ($p<0.05$) difference at 30 min between the 200 and 400 mg/kg VA ethanol extract treatment groups (60–180 min interval) was also observed.

The pharmacological potential exhibited by VA may be a result of its rich phytoconstituents, including flavonoids, alkaloids, triterpenoids, saponins, steroids, tannins, etc.[31,41] VA has been characterized previously using HPLC,[64] and has been reported to be rich in alkaloids, including oxoassoanine, piperine, piperidine, nitrosodimethylamine, N-propylamine, dihydro-oxo-demethoxyhaemanthamine, crinane-3-alpha-ol, and nitrosodimethylamine. Alkaloids, which are a class of naturally occurring phytochemicals, are found in many species of plants, and in several studies, they have been reported as one of the major phytochemicals involved in the analgesic efficacy of numerous plants.[65,66,67] Previous studies have identified several mechanisms by which alkaloids can exert their pain-relieving properties.[68] Opioid receptor activation, ion channel modulation, pro-inflammatory cytokine inhibition, and neurotransmitter modulation, such as serotonin and norepinephrine, are some of these mechanisms. [69,70]

VA, a plant rich in alkaloids, holds promise as a potential agent for pain alleviation. It is critical to gain a better understanding of the specific interactions between the alkaloids found in VA and molecular targets associated with pain pathways. Exploring the pharmacokinetics and pharmacodynamics of VA alkaloids will help define their absorption, distribution, metabolism, and excretion in the body, allowing for a more in-depth understanding of the plant's analgesic properties and the development of targeted therapeutic interventions for pain management.

Table 3: Effect of Ethanol Extract of VA on Acetic Acid-Induced Writhing

Treatment Group	Dose (mg/kg)	Average Number of Writhing	Inhibition (%)
Group I	Normal Saline	22.63 ± 1.45	0
Group II	40 mg/kg Aspirin	8.67 ± 0.88^{a}	61.69
Group III	200 mg/kg *VA*	14.75 ± 0.46^{bc}	34.82
Group IV	400 mg/kg *VA*	12.84 ± 1.06^{ac}	43.62
Group V	800 mg/kg *VA*	9.33 ± 1.86^{a}	58.77

Results are presented as mean ± SEM (n=4). a – means significant increase at $p<0.001$ when compared with group I; b – means significant increase at $p<0.01$ when compared with group I; c –means significant increase at $p<0.005$ when compared with group II

Table 4: Analgesic Effect of Ethanol Extract from VA on Latency to Hot Plate Test in Rats

Treatment Group	Latency(s)				
	0 mins	30 mins	60 mins	120 mins	180 mins
Normal Saline	4.43 ± 0.53	3.20 ± 0.25	4.35 ± 0.33	3.76 ± 1.07	2.98 ± 0.06
Diclofenac (25 mg/kg)	3.86 ± 0.02	4.97 ± 0.04^{b}	6.98 ± 0.02^{a}	9.93 ± 0.12^{a}	8.08 ± 0.42^{a}
VA (200 mg/kg)	3.62 ± 0.38	3.06 ± 0.05^{d}	5.28 ± 0.09^{d}	5.05 ± 0.04^{bd}	4.27 ± 0.10^{bc}
VA (400 mg/kg)	4.01 ± 0.22	5.73 ± 0.06^{ad}	7.15 ± 0.22^{a}	6.96 ± 0.09^{ad}	8.10 ± 0.09^{a}
VA (800 mg/kg)	3.94 ± 0.06	5.27 ± 0.17^{a}	7.22 ± 0.03^{a}	8.50 ± 0.14^{a}	± 0.25^{a}

Results are presented as mean ± standard error of mean (n = 4). a – means significant increase at $p<0.001$ when compared with group I; b – means significant increase at $p<0.01$ when compared with group I; c - means significant increase at $p<0.001$ when compared with group II; d – means significant increase at $p<0.05$ when compared with group II.

Table 5: Analgesic Effect of Ethanol Extract from VA (VA) on Tail-Flick latency in Rats

Treatment Group	Response (Seconds)				
	0 mins	30 mins	60 mins	120 mins	180 mins
Normal Saline	1.93 ± 0.36	2.09 ± 0.52	2.04 ± 0.03	2.23 ± 0.74	2.18 ± 1.02
Diclofenac (25 mg/kg)	2.14 ± 0.10	2.48 ± 1.02^{b}	4.33 ± 0.26^{a}	5.28 ± 0.08^{a}	5.91 ± 0.31^{a}
VA (200 mg/kg)	2.06 ± 0.08	2.31 ± 0.07^{cf}	2.94 ± 0.42^{bd}	3.66 ± 0.12^{bd}	3.82 ± 0.03^{be}
VA (400 mg/kg)	2.11 ± 1.05	2.43 ± 0.16^{b}	3.96 ± 1.08^{af}	4.73 ± 0.02^{af}	4.96 ± 0.12^{af}
VA (800 mg/kg)	2.24 ± 0.12	2.51 ± 0.36^{b}	4.31 ± 0.33^{a}	± 0.04^{a}	5.77 ± 0.30^{a}

Results are presented as mean ± standard error of mean (n = 4). a – *means significant increase at p<0.001 when compared with group I; b – means significant increase at p<0.01 when compared with group I; c - means significant increase at p<0.05 when compared with group I; d – means significant increase at p<0.001 when compared with group II; e – means significant increase at p<0.01 when compared with group II; f – means significant increase at p<0.05 when compared with group II.*

Conclusion

In all test models used in this investigation, *Vernonia amygdalina* (VA) was found to have analgesic effects. These analgesic effects were time-dependent, comparable to conventional medications (Diclofenac and Aspirin), with a quick onset within 30 minutes and prolonged analgesia lasting up to 180 minutes, especially at higher doses of VA (400 and 800 mg/kg). The plant has potent phytochemicals such as alkaloids that may alter how pain is perceived through activating opioid receptors and having anti-inflammatory properties. VA has the potential to be a natural pain reliever, but more research is needed, with a focus on safety profiles and dosage optimization for clinical applications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Clark LM, Wrona S. A Sensory and Emotional Experience: Pediatric Chronic Pain & Mental Health Pain Manag Nurs. 2016; 17: 97
2. Perrot S, Cohen M, Barke A, Korwisi B, Rief W, Treede R-D. The IASP classification of chronic pain for ICD-11: chronic secondary musculoskeletal pain. Pain. 2019; 160: 77–82.
3. Craig KD, MacKenzie NE. What is pain: Are cognitive and social features core components? Paediatr Neonatal Pain. 2021; 3: 106–118.
4. Luo Y, Wang C-Z, Sawadogo R, Tan T, Yuan C-S. Effects of Herbal Medicines on Pain Management. Am J Chin Med. 2020; 48: 1–16.
5. Williams AC de C, Craig KD. Updating the definition of pain. Pain. 2016; 157: 2420–2423.
6. Toth LA. Interacting Influences of Sleep, Pain, and Analgesic Medications on Sleep Studies in Rodents Comp Med. 2019; 69: 571–578.
7. McCann C. Preoperative analgesia for children and adolescents to reduce pain associated with dental treatment. Evid Based Dent. 2017; 18: 17–18.
8. Panayotova GS, Dimitrov DA. Modeling from Time Series of Complex Brain Signals. Int J Signal Process Syst. 2021; 9: 1–6.
9. Lim RKS, Armstrong D, Pardo EG. Pharmacology of Pain, Vol. 9. Pergamon Press: Elsevier; 2016. 250p.
10. Vanegas H, Vazquez E, Tortorici V. NSAIDs, Opioids, Cannabinoids and the Control of Pain by the Central Nervous System Pharmaceuticals. 2010; 3: 1335–1347.
11. Gargya I, Singh B, Talnia S. NSAIDS (Non- Steroidal Anti-Inflammatory Drugs)- Their Effects and Side Effects in Orthodontic Therapy- A Review Dent J Adv Stud. 2017; 05: 8–13.
12. Fokunang C. Overview of non-steroidal anti-inflammatory drugs (NSAIDs) in resource limited countries. MOJ Toxicol. 2018; 4(1): 5–13.
13. Drini M. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Aust Prescr. 2017; 40(3): 91-93.
14. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochem Pharmacol. 2020; 180: 114147
15. Jahnavi K, Pavani Reddy P, Vasudha B, Narender B. Non-steroidal anti-inflammatory drugs: an overview. J Drug Deliv Ther. 2019; 9: 442–448.
16. Kumar P, Shah S, Gupta A. Emerging role of cannabinoids and synthetic cannabinoid receptor 1/cannabinoid receptor 2 receptor agonists in cancer treatment and chemotherapy-associated cancer management. J Cancer Res Ther. 2021; 17: 1-9.
17. Reed MD. The Balance Between Effective Opioid-Based Pain Management and Patient Safety: Can It Be Achieved? J Pediatr Pharmacol Ther. 2013; 18: 264–268.
18. Mauritz MD, Hasan C, Dreier LA, Schmidt P, Zernikow B. Opioid-Induced Respiratory Depression in Pediatric Palliative Care Patients with Severe Neurological Impairment—A Scoping Literature Review and Case Reports. Children. 2020; 7: 312.
19. Anne S, Finestone SA, Paisley A, Monjur TM. Plain Language Summary: Opioid Prescribing for Analgesia After Common Otolaryngology Operations. Otolaryngol Head Neck Surg. 2021; 164: 704–711.
20. Chen H, Chen H, Wang C, Gong Y, Xiang X, Zhang H. Current application of commonly-used traditional Chinese herbal medicine and formulae for preventing severe infectious diseases. Pharm Care Res. 2020; 20: 291–295.
21. Mohankumar R, Prakash SEL, Irfan N, Mohanraj S, Kumarappan C. Evaluation of analgesic, anti-inflammatory, and antipyretic activities of *Ziziphus mauritania* Lam leaves in animal models. Pharmacol Res - Mod Chin Med. 2022; 4: 100153.
22. Zhou F, Lan K, Li X, Mei Y, Cai S, Wang J. The complete chloroplast genome sequence of *Vernonia amygdalina* Delile. Mitochondrial DNA B Resour. 2021; 6: 1134–1135.
23. Nguyen PAT, Khang DT, Nguyen PTT, Do HDK. The complete chloroplast genome of *Elephantopus scaber* L. (Vernonioideae, Asteraceae), a useful ethnomedicinal plant in asia. Mitochondrial DNA B: Resour. 2023; 8(9): 936–941
24. Liu X, Yang R, Xu Q, Zhou M, Feng J, Wang G, Lin T, Tian W, Chen H. Tautomeric phytosterols from *Vernonia amygdalina* Delile and their anti-cervical cancer activity. Bioorg Chem. 2022; 128: 106068.
25. Burkill HM. The useful plants of west tropical Africa, Vols. 1-3. (2nd ed.). Kew: Royal Botanical Garden; 1995.
26. Adejumo TO, Vögele RT. Biopesticides. Berlin: Logos Verlag Berlin GmbH; 2021. 300p.
27. Oladele JO, Oyeleke OM, Oladele OT, Olaniyan M. Neuroprotective mechanism of *Vernonia amygdalina* in a rat model of neurodegenerative diseases. Toxicol Rep. 2020; 7: 1223–1232.
28. Alozie EU, Iheanacho KM, Alisi CS, Asiwe ES, Iweala E. Comparative Hepatoprotective Activity of *Vernonia amygdalina* Leaf Solvent Extracts in Alloxan-Induced Diabetic Rats. Trop J Nat Prod Res. 2023; 7(7): 3517-3523.
29. Koffi JA, Silué KD, Tano DK, Dable TM, Yavo W. Evaluation of in vitro antiplasmodial effect of extracts from seven medicinal plants used to treat malaria in Côte d'Ivoire. BioImpacts. 2020; 10: 151–157.
30. Olamoyegun MA, Omisore NO, Yusuf AO, Odewale TE. Assessment of Pharmacodynamic Interactions and Toxicological Effects of *Vernonia amygydalina* –Metformin Co-Administration on Streptozotocin-Induced Diabetic Wistar Rats. Trop J Nat Prod Res. 2022; 6 (12): 2073-2080.
31. Adedayo BC, Ajiboye OM, Oyeleye IS, Ojo RO, Oboh G. Effect of alkaloid extract from *Andrographis paniculata* (Burm. f.) Nees and *Phyllanthus amarus* Schumach. & Thonn. on cognitive related biochemicals in the brain of streptozotocin-induced diabetic rats. Pharmacol Res - Mod Chin Med. 2023; 9: 100314.
32. Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. Anti-inflammatory activities of ethanol extract of *Carica papaya* leaves. Inflammopharmacology. 2008; 16: 168–173.
33. Koster R. Acetic acid for analgesics screening. In: Fed Proc. 1959; 18: 412-417.
34. Shehu A, Olurishe TO, Zezi AU, Ahmed A. Acute Toxicological, Analgesic and Anti-Inflammatory Effects of

Methanol Extract of *Laggera aurita* Linn F (Compositae) in Mice and Rats. Afr J Pharmacol Ther. 2016; 5(2): 65-73.
35. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines. J Pharmacol Exp Ther. 1953; 107(3): 385-393.
36. Hayashi G, Takemori AE. The type of analgesic-receptor interaction involved in certain analgesic assays. Eur J Pharmacol. 1971; 16: 63–66.
37. Onasanwo S, Oyebanjo O, Ajayi A, Olubori M. Mechanisms of action of the anti-nociceptive and anti-inflammatory effects of leaf extract of *Vernonia amygdalina*. J Intercult Ethnopharmacol. 2017; 6(1): 192-198.
38. Adiukwu PC, Kayanja FIB, Rugera S, Murokore B, Nambatya GK, Ezeonwumelu JOC, Tanayen JK, Murokore BJ, Twikirize O, Twinomujuni S, Byamugisha D, Imanirampa L. Antipyretic and antinociceptive properties of the aqueous extract and saponin from an edible vegetable: *Vernonia amygdalina* leaf. Afr J Food Agric Nutr Dev. 2013; 13: 7587–7606.
39. Alara OR, Abdurahman NH, Abdul Mudalip SK, Olalere OA. Phytochemical and Pharmacological Properties of *Vernonia amygdalina*: A Review J Chem Eng Ind Biotechnol. 2017; 2: 80–96.
40. Njan AA, Adzu B, Agaba AG, Byarugaba D, Díaz-Llera S, Bangsberg DR. The Analgesic and Antiplasmodial Activities and Toxicology of *Vernonia amygdalina*. J Med Food. 2008; 11: 574–581.
41. Purnamasari O, Azizah Z, Misfadhila S. Phytochemical and Pharmacological Activities of African Leaf (*Vernonia Amygdalina*): A Review. Int J Res Pub Rev. 2023; 4: 1752–1758.
42. Abubakar A, Nazifi A, Hassan F, Duke K, Edoh T. Safety assessment of *Chlorophytum alismifolium* tuber extract (Liliaceae): Acute and sub-acute toxicity studies in Wistar rats. J. Acute Dis. 2019; 8: 21-27.
43. Rahman SMM, Atikullah Md, Islam MdN, Mohaimenul Md, Ahammad F, Islam MdS, Saha B, Rahman MdH. Anti-inflammatory, antinociceptive and antidiarrhoeal activities of methanol and ethyl acetate extract of *Hemigraphis alternata* leaves in mice. Clin Phytoscience. 2019; 5:16.
44. Olajumoke E, Ibrahim O, Akinwunmi A, Viola N. Acute Toxicity of Aqueous Leaf Extract of *Euphorbia heterophylla* L. in Sprague Dawley Rats. J Complement Med Res. 2016; 1: 1–10.
45. Lala V. Liver Function Tests. StatPearls - NCBI Bookshelf 2023. https://www.ncbi.nlm.nih.gov/books/NBK482489/.
46. Cantalapiedra-Hijar G, Abo-Ismail M, Carstens GE, Guan LL, Hegarty R, Kenny DA, McGee M, Plastow G, Relling A, Ortigues-Marty I. Review: Biological determinants of between-animal variation in feed efficiency of growing beef cattle. Animal. 2018; 12: 321–335.
47. Eslam M, El-Serag HB, Francque S, Sarin SK, Wei L, Bugianesi E, George J. Metabolic (dysfunction)-associated fatty liver disease in individuals of normal weight. Nat Rev Gastroenterol Hepatol. 2022; 19: 638–651.
48. Esmaeili N, Carter CG, Wilson R, Walker SP, Miller MR, Bridle AR, Symonds JE. Proteomic investigation of brain, liver and intestine in high feed intake and low feed intake Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 2022; 551: 737915.
49. Kumar A, Kaur H, Singh A. Neuropathic Pain models caused by damage to central or peripheral nervous system. Pharmacol Rep. 2018; 70: 206–216.
50. Saganuwan SA. Piroxicam: Source for Synthesis of Central Nervous System (CNS) Acting Drugs. Cent Nerv Syst Agents Med Chem. 2017; 17(2): 135-140.
51. Mazo I, Roza C, Zamanillo D, Merlos M, Vela JM, Lopez-Garcia JA. Effects of centrally acting analgesics on spinal segmental reflexes and wind-up. Eur J Pain 2014; 19: 1012–1020.
52. Martini M, Perez-Marcos D, Sanchez-Vives MV. Modulation of pain threshold by virtual body ownership. Eur. J. Pain. 2014; 18: 1040–1048.
53. Kress HG. Tapentadol and its two mechanisms of action: Is there a new pharmacological class of centrally-acting analgesics on the horizon? Eur J Pain. 2010; 14: 781–783.
54. Martínez V, Abalo R. Peripherally acting opioid analgesics and peripherally-induced analgesia. Behav Pharmacol. 2020; 31: 136–158.
55. Stein C. Targeting pain and inflammation by peripherally acting opioids. Front. Pharmacol. 2013; 4.
56. Sehgal N. Peripherally Acting Opioids and Clinical Implications for Pain Control. Pain Physician. 2011; 14: 249–258.
57. Rohdewald P, Neddermam E, Handwerker HO. Dose dependency of the analgesic effect of metamizole to painful tooth pulp stimulation. Pain. 1987; 30: S53.
58. Patil A, Konda VCR. Dose-dependent analgesic activity of mexiletine on thermally induced pain in rats. Int J Basic Clin Pharmacol. 2018; 8: 90-94.
59. Rai N, Vyas S, Phadnis P. Evaluation of analgesic activity of *Ficus racemosa* leaf extract using acetic acid induced writhing method in mice. Int J Basic Clin Pharmacol. 2016: 60–64.
60. Jo H-G, Lee G-Y, Baek CY, Song HS, Lee D. Analgesic and Anti-Inflammatory Effects of *Aucklandia lappa* Root Extracts on Acetic Acid-Induced Writhing in Mice and Monosodium Iodoacetate-Induced Osteoarthritis in Rats. Plants 2020; 10: 42.
61. Olela B, Mbaria J, Wachira T, Moriasi G. Acute Oral Toxicity and Anti-inflammatory and Analgesic Effects of Aqueous and Methanol Stem Bark Extracts of *Piliostigma thonningii* (Schumach.). Evid Based Complement Alternat Med. 2020; 2020: 1–10.
62. Sharma S, Khare S, Dubey BK, Joshi A, Jain A. Analgesic activity of poly herbal formulation in experimental rats by acetic acid induced writhing test model and Hot plate model. J Drug Deliv Ther. 2019; 9(Suppl. 2): 276-280.
63. Alzahrani AR, Shahid I. Determination of Analgesic Potential of Ethanol Extract of *Brassica campestris* Leaves in Rats. Pharmacognosy Res. 2022; 14: 297–303.
64. Oboh G, Adedayo BC, Adetola MB, Oyeleye IS, Ogunsuyi OB. Characterization and neuroprotective properties of alkaloid extract of *Vernonia amygdalina* Delile in experimental models of Alzheimer's disease. Drug Chem Toxicol. 2020; 45: 731–740.
65. Shareef H, Naeem S, Zaheer E. Comparative Analgesic Activity of Selected Medicinal Plants from Pakistan: Analgesics from plants. Proc Pak Acad Sci B. 2019; 56(3): 57-67.
66. Tian B, Tian M, Huang S-M. Advances in phytochemical and modern pharmacological research of *Rhizoma corydalis*. Pharm Biol. 2020; 58: 265–275.
67. Wang M, Zhang X-M, Fu X, Zhang P, Hu W-J, Yang B-Y, Kuang H-X. Alkaloids in genus *stephania* (Menispermaceae): A comprehensive review of its ethnopharmacology, phytochemistry, pharmacology and toxicology. J Ethnopharmacol. 2022; 293: 115248.
68. Le HM, Huynh NT, Vo TT, Nguyen TT, Nguyen QM. Evaluation of Pain-relieving effect of the herbal remedy "Hoang Ky Que Chi Ngu Vat Thang" in Animal Model. Trop J Nat Prod Res. 2023; 7(6): 3153-3157.
69. Jiang W, Tang M, Yang L, Zhao X, Gao J, Jiao Y, Li T, Tie C, Gao T, Han Y, Jiang J-D. Analgesic Alkaloids Derived from Traditional Chinese Medicine in Pain Management. Front Pharmacol. 2022; 13: 851508.
70. Feng J, Chen K, Shen S, Luo Y, Liu X, Chen X, Gao W, Tong Y-R. The composition, pharmacological effects, related mechanisms and drug delivery of alkaloids from *Corydalis yanhusuo*. Biomed Pharmacother. 2023; 167: 115511.