Original Research Article

Analgesic Effects of Methanol Leaf Extracts from *Datura alba ness* in Male Wistar Rats ThroughReduction of C-Reactive Protein Levels

ABSTRACT

Introduction: The increasing interest in utilizing natural products from medicinal plants for pain management stems from their wide availabilityand minimal side effects. This study aims to evaluate the toxicity profile of *Datura alba ness* (DAN) extracts and assess their potential analgesic activity through preventive and therapeutic analgesia models in Wistar rats, as well as to investigate the possible mechanisms of action using molecular docking.

Materials and Methods: Fresh leaves of DAN were collected from a garden in the Abua/Odua Local Government Area of Rivers State, Nigeria. The leaves wereextracted using cold maceration to obtain ethanolic extracts of *Datura alba ness* (MEDAN). Twelve mice (19-30g) were used for toxicity study, while sixty Wistar rats(140-180g) were used for analgesic studies. Serum C-reactive protein levels were determined using ELISA methods, and molecular docking simulations were conducted using AutoDock and Discovery Studio Visualizer.

Results: The results indicated that MEDAN was very safe, with an LD_{50} of 5000 mg/kg. The findings demonstrate that MEDAN significantly increased the pain threshold in male Wistar rats after three weeks of administration in a dose-dependent manner during the preemptive analgesia study, while pain thresholds increased within one hour but decreased over time in the therapeutic analgesia study. The combination of MEDAN with a standard drug (Flexicam) resulted in improved analgesic effects at the 500 mg/kg dose. Additionally, the study revealed that MEDAN significantly reduced serum C-reactive protein levels after three weeks of oral administration. Molecular docking studies indicated that stigmasterol, gamma-sitosterol, and cholest-5-en-3-ol, 24-propylidene-(3β) from MEDAN demonstrated binding affinity to C-reactive protein receptors, mimicking the effects of the reference drug, celecoxib.

Conclusion: This study concludes that MEDAN leaves exhibit significant efficacy in pain reduction by lowering C-reactive protein levels. This effect was especially enhanced when the 500 mg/kg dose of MEDAN was combined with the standard drug, Flexicam. The findings provide a scientific basis supporting the traditional medicinal use of *Datura alba ness* for pain management.

Keywords: *Datura alba ness*, analgesia, c-reactive protein, molecular docking

INTRODUCTION

Pain is defined as an "unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage." The pain perception threshold refers to the point at which an individual first perceives pain, and this threshold exhibits minimal variability among individuals [1, 2]. Pain signals are transmitted through a complex network of pathways from peripheral receptors to the central nervous system. Primary afferent pain fibres synapse with second-order neurons in the dorsal horn of the spinal cord, with ascending spinothalamic and spinoreticular tracts carrying pain signals to the brain. These signals are processed in the thalamus and relayed to the cortex for interpretation. Pain modulation occurs through descending tracts involving the periaqueductal grey matter and nucleus raphe magnus. Neuropathic pain arises from nerve damage, with proposed mechanisms involving both peripheral and central sensitization pathways [1, 3]. Pain is classified based on various criteria, including the affected body region (somatic or visceral pain), duration (acute or chronic), causative factors (neuropathic or inflammatory), and the mechanism of transmission (central, peripheral, or nociceptive) [2-4]. Analgesics are drugs used in pain management. They exert their effects through various mechanisms which highlight the diverse approaches to pain management. For example, acetaminophen (paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDs) relieve pain by inhibiting cyclooxygenase enzymes (COX) in the brain, reducing pain and fever while opioid receptors act by inhibiting the pain pathway and general modulation and dampening of pain perception [5-7].

Traditional African medicine primarily relies on botanical preparations for the treatment of illnesses in both animals and humans. In Traditional African Herbal Medicine (TAHM), remedies are created by extracting components from various parts of plants, including roots, barks, leaves, flowers, seeds, and aerial parts, either as single entities or through combinations of different parts from various plant species [8, 9]. Research aimed at validating traditional knowledge has demonstrated the efficacy of TAHM preparations in alleviating pain. Bioactive compounds have been identified in certain plant species, and further research could lead to the acceptance and integration of some traditional pain treatments into modern medical practices. This exploration may pave the way for discovering new bioactive compounds that could be developed into evidence-based pharmaceutical medications [10, 11]. Some of the African plants screened for their potential analgesic activity include *Acacia modesta*[12], *Alstoniaboonei*[13], *Annona muricata*[14], *Bryophyllumpinnatum*[15], *Chromolaenaodorata*[16], *Hymenocardiaacida*[17], *Ipomoea involucrate*[18], *MyristicaFragransHoutt*[19], *Tamarindusindica*[20],*Zea mays*and many others [21].

The Datura genus is generally characterized as comprising annual or perennial herbs and shrubs that are part of the Solanaceae family. The leaves have petioles, and the tubular calyx splits around near the base, while the funnel-shaped, elongated corolla features cuspidate lobes [22, 23]. The seeds are dark, flat, and kidney-shaped, while the spiny fruits resemble walnuts, giving

rise to the name "thorn apple." The plant is also commonly known as Jimsonweed or Devil's trumpet [23, 24]. *Datura alba ness* (DAN) is one of the most prevalent species in Nigeria. It is commonly known as *Zakami*, while the Ogoni and Ndokwa tribes refer to it as *Jegemi* and *Gegemu*, respectively [25]. Despite its toxic properties, *Datura* has long been used in traditional medicine as an anaesthetic and for treating bruises, wounds, and skin ulcers [25, 26]. Our earlier report on the proximate, phytochemical and bioactive analysis of *Datura alba ness*leaf extract showed it contains various bioactive compounds with potential medicinal properties [25]. Datura species, *Daturafastuosa* and *Daturametel*, have been shown to exhibit strong analgesic properties[27, 28]. However, no research has yet explored the potential analgesic effects of *Datura alba ness* (DAN), despite anecdotal evidence suggesting that brewing its leaves in local gin may help manage pain. This study aims to evaluate the potential analgesic activity of DAN extracts using preventive and therapeutic analgesia models in Wistar rats and the possible mechanism of action using molecular docking.

MATERIAL AND METHODS

Collection, Identification, preparation and extraction of plant materia

Fresh leaves of *Datura alba ness* were collected from a garden in the Abua/Odua Local Government Area of Rivers State, Nigeria. The plant was identified and authenticated by a taxonomist from the Department of Plant Science and Biotechnology at the University of Port Harcourt and assigned the voucher number UPH/295. The leaves were washed and air-dried at room temperature for two weeks until they became crisp. They were then ground into coarse particles. The extraction process followed a cold maceration technique as previously described[29, 30]. Approximately 400 g of the coarsely ground *Datura alba ness* leaves were soaked in 2000 ml of methanol and left to stand for 72 hours with occasional stirring. The macerated mixture was then filtered using filter paper, and the filtrate was evaporated in a water bath at 50°C to yield the methanol extract of *Datura alba ness* (MEDAN).

Research Animals

Twelve (12) mice weighing between 19 and 30 grams, along with sixty (60) Wistar rats weighing between 140 and 180 grams, were obtained from the animal house at the Faculty of Basic Medical Sciences, University of Port Harcourt, for the study. All animal experiments adhered to the principles of laboratory animal care[31]. The animals were given a 14-day acclimatization period at the experimental site before the study began. They were housed in wooden cages under standard conditions, with a temperature of 22±3°C, 12 hours of light, and 12 hours of darkness. Throughout the study, they had access to standard laboratory rat chow *ad libitum*. At the commencement of the experiment, all MEDAN administration was done orally.

Acute Toxicity Test

Twelve (12) were used to assess acute toxicity following Lorke's method[32]. The test was conducted in two phases. In the first phase, nine (9) mice were divided into three (3) groups of

three (3) mice each.Groups 1, 2, and 3 were administered 10, 100, and 1000 mg/kg of MEDAN, respectively, to determine the dose range that might cause toxic effects. In the second phase, the remaining three (3) mice received 1600, 2900, and 5000 mg/kg of MEDAN. They were monitored for 24 hours for signs of weakness or mortality. The LD_{50} was calculated to be 5000 mg/kg of MEDAN.

Preemptive and Therapeutic Analgesia Tests

For the preemptive analgesia test, Wistar rats were divided into six groups, with five animals in each group. Group I, the negative control, received distilled water, while Group II, the positive control, was given the standard pain medication Flexicam (1.5 mg/kg) (Hovid, Malaysia). Groups III and IV were administered 500 mg/kg and 1000 mg/kg of MEDAN, respectively. Groups V and VI received 500 mg/kg and 1000 mg/kg of MEDAN in combination with the standard drug. Both MEDAN and the standard drug were administered orally for two weeks before the experiment. This test was carried out on day 15, 17 and 19th. The therapeutic analgesia test followed a similar design to the preemptive test involving sixty Wistar and grouped six (6) groups of five (5) animals treated with MEDAN and standard drug in the same manner as the preemptive analgesia test, however, the pain was first induced using an analgesimeterbefore treatment with extracts and standard drugs.

AnalgesimeterTest

This experiment followed the previously described method [33, 34]. The rat was suspended vertically, and its left hind paw was placed between the plinth and the finger of the Ugo Basileanalgesimeter (Ugo Basile 7200, Italy). The machine measured pain thresholds by applying increasing pressure to the hind paw. The force at which each rat withdrew its paw was recorded. This was considered the pain threshold and expressed as force per gram (f/g). The recording of pain threshold was done 15, 17 and 19th days for the preemptive analgesia and 1, 2 and 4 hours post-treatment for the therapeutic analgesia test.

Sample Collection and Analysis

After the study, blood samples were obtained from the recto-orbital sinus and placed in a plain sample bottle. The blood was allowed to sit for 30 minutes before being centrifuged at 3000 rpm for 5 minutes. The supernatant serum was carefully decanted using a micropipette and stored at 2^{o}C until laboratory analysis. The concentration of C-reactive protein in the serum was measured using standard laboratory test kits (Elabscience, China) via the ELISA method.

InsilcoMolecular Docking

This method combines computer science and computational methods with experimental biology by simulating and predicting molecular interactions, gene regulation and drug targets[35, 36]. The crystal structure of C-reactive protein (CRP) was retrieved from the Protein Data Bank and prepared using AutoDock v4.2 (Scripps Research Institute, USA) by removing ligands and

water, adding missing hydrogens, and saving the structure in PDBQT format. Ligands were docked to various protein targets, and binding affinities were evaluated using AutoDockVina. The docking grid for CRP was established, and cluster analysis based on RMSD values identified the most reliable binding conformations. Compounds with binding affinities greater than the standard antimalarial (artesunate) were visualized with Discovery Studio Visualizer (BIOVIA, USA).

Statistical Analysis

Data analysis was performed using GraphPad Prism version 8.0.1 (244). One-way analysis of variance (ANOVA) and post hoc tests were conducted to assess mean differences. The results are presented as means and standard errors of the mean (M ± SEM), with statistically significant values indicated at $p < 0.05$ within a 95% confidence interval.

RESULTS

Table 1: Preventive effects of methanolic leave extract of *Datura alba ness* on pain threshold in male *Wistar* rats usingan analgesimeterafter 3 weeks of administration

Research Groups	Pain Threshold (f/g)		
	Day15	Day17	Day 19
Negative control (distilled water)	142.50±2.79	208.50±6.34	165.00±2.34
Positive control (Felxicam 1.5 mg/kg)	148.00±1.89	222.10±2.92	239.00±2.58
500mg/kgMEDAN	172.30±4.24^{ab}	155.10±4.55^{ab}	166.00±2.82^{b}
1000mg/kgMEDAN	227.50±4.85^{ab}	190.70±3.50^{b}	222.80±3.65^{ab}
500mg/kg MEDAN +Felxicam	114.30±1.71^{ab}	228.10±3.43^{a}	261.80±3.83^{ab}
1000mg/kgMEDAN +Felxicam	165.60±5.31^{ab}	169.20±2.62^{ab}	184.00±4.57^{ab}

Data are expressed as Mean ± SEM

$p< 0.05^{a}$: significant compared to negative control (Group 1)

$p< 0.05^{b}$: significant compared to Standard drug (Felxicam) *(Group 2)*

Table 1 presents the pain threshold in male Wistar rats following three weeks of administration of methanolic leaf extract of *Datura alba ness* (MEDAN). The data indicate that MEDAN at doses of 500 and 1000 mg/kg significantly increased the pain threshold on day 15 in a dose-dependent manner compared to both negative and positive controls ($p < 0.05$). However, when combined with Flexicam, the 500 mg/kg MEDAN dose resulted in a significantly reduced pain threshold, while the 1000 mg/kg MEDAN dose improved the pain threshold.On day 17, there was a significant decrease in pain threshold for rats treated with 500 and 1000 mg/kg MEDAN ($p < 0.05$), which was reversed when MEDAN was combined with a standard drug, leading to significantly higher pain thresholds for both dosages. By day 19, the increased pain threshold persisted in rats treated with 1000 mg/kg MEDAN, and further improvement was observed when the 500 mg/kg dose was combined with the standard drug.These findings suggest that *Datura*

alba ness exhibits potential analgesic effects, which may be enhanced when used in conjunction with standard pain relief medications.

Table 2:Therapeutic effects of methanolic leaves extract of *Datura alba ness* on pain threshold in male *Wistar* rats.

Research Groups	Pain Threshold (f/g)		
	After 1 hr	After 2 hrs	After 4hrs
Negative control (distilled water)	145.70±2.72	170.40±3.32	212.90±3.45
Positive control (Felxicam 1.5 mg/kg)	87.33±2.85	243.90±3.68	257.10±4.35
500mg/kgMEDAN	206.50±4.80^{ab}	162.90±4.84^{b}	230.10±3.87^{ab}
1000mg/kgMEDAN	163.30±4.13^{ab}	172.10±2.50^{b}	203.50±2.37^{b}
500mg/kg MEDAN +Felxicam	238.80±5.01^{ab}	169.90±3.56^{b}	208.00±3.88^{b}
1000mg/kgMEDAN +Felxicam	124.30±2.01^{ab}	160.00±3.13^{b}	250.30±4.06^{a}

Data are expressed as Mean ± SEM
$p< 0.05^{a}$: significant compared to negative control (Group 1)
$p< 0.05^{b}$: significant compared to Standard drug (Felxicam) *(Group 2)*

Table 2 presents the changes in pain threshold in male Wistar rats following oral administration of lead extracts of Datura alba Ness (MEDAN). The results show that doses of 500 and 1000 mg/kg of MEDAN, as well as 500 mg/kg MEDAN in combination with a standard drug, resulted in an increased pain threshold onehour post-administration. However, after two hours, this effect diminished, as all MEDAN doses and their combinations with the standard drug led to significantly lower pain thresholds compared to the standard drug ($p < 0.05$).After four hours, 500 mg/kg MEDAN caused an increase in pain threshold compared to both the negative control and the standard drug, while 1000 mg/kg MEDAN significantly decreased the pain threshold in comparison to the standard drug. When combined with the standard drug, both 500 and 1000 mg/kg MEDAN resulted in a significant reduction in pain threshold compared to the standard drug and the negative control, respectively.

Table 3: Effects of methanolic leaf extract of *Datura alba ness* on serum level C Reactive Protein of male *Wistar* rats after three weeks of administration.

Research Groups	C-Reactive Protein(mg/l)
Negative control (distilled water)	266 ± 3.81
Positive control (Felxicam 1.5 mg/kg)	380± 6.52
500mg/kgMEDAN	298 ± 7.08^{b}
1000mg/kgMEDAN	300± 17.8^{b}
500mg/kg MEDAN +Felxicam	278 ± 3.53^{b}
1000mg/kgMEDAN +Felxicam	279 ± 2.20^{b}

Data are expressed as Mean ± SEM
$p< 0.05^{a}$: significant compared to negative control (Group 1)

p< 0.05[b]: significant compared to Standard drug (Felxicam) *(Group 2)*

Table 3 illustrates the effects of a three-week oral administration of methanolic leaf extract of *Datura alba ness* (MEDAN) on serum C-reactive protein levels. The data reveal that all MEDAN doses and their combinations with a standard drug significantly reduced the mean levels of C-reactive protein compared to the standard drug ($p < 0.05$). However, no changes were observed when compared to the negative control.

Table 4: Insilico molecular docking chart showing binding affinity of compounds to CRP using celecoxib as the reference compound

S/N	Compound Name	GCMC Area%	GCMC Height%	GCMC H/A mark	Bat Ref Drug C-pr	Bat Cpd C-pr	Lipinski Violation
R	Celecoxib				-9.0		
1	Dodecanoicacid, 1,2,3-propanetriyl ester	4.32	2.21	5.07			2
2	Stigmasterol	3,11	2.74	3.01		-9.2	1
3	Gamma sitosterol	10.77	7.98	3.57		-9.2	1
4	Cholest-5-en-3-ol, 24-propylidene-, (3β)	2.18	1.40	4.12		-9.7	1

Table 4 shows thebinding Affinity of compounds to CRP using celecoxib as the reference compound, the binding of the C=reactive protein to the reference drug is -9.0 out of the 29 compounds from the extract[25]. The following compound showed a binding affinity with the CPR receptor. Dodecanoic acid, 1,2,3-propanediol ester, Stigmasterol, gamma sitosterol and Cholest-5-en-3-ol, 24-propylidene-, (3β).

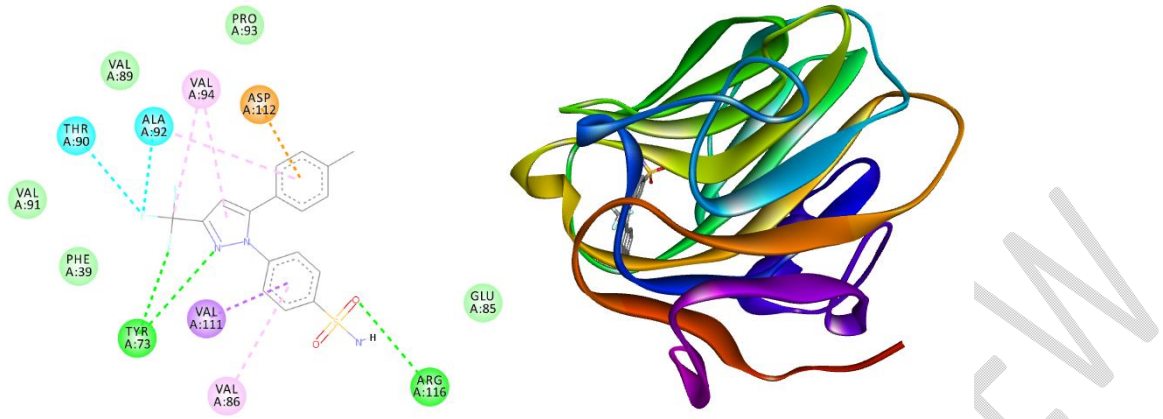

Figure 1: 2D and 3D view of the interaction between celecoxib and amino acid in the binding site of c-reactive protein

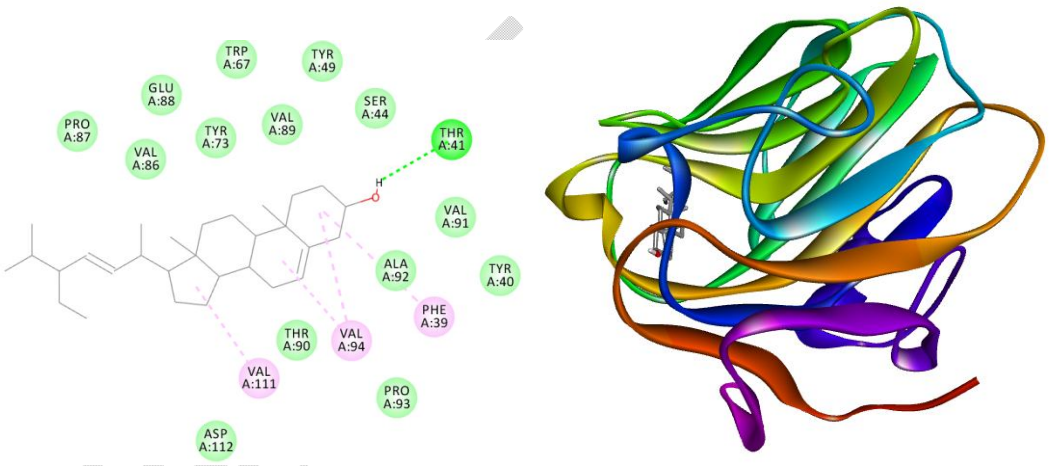

Figure 2: 2D and 3D view of the interaction between stigmasterol and amino acid in the binding site of c-reactive protein respectively

DISCUSSION

Traditional medicine in Africa and Asia relies on the use of leaves, roots, seeds, and stems from various plants to treat different ailments. Numerous studies have sought to validate the effectiveness of some of these plants for pain management, leading to the identification of bioactive compounds that could be developed into standardized pharmaceuticals. Additionally, the occurrence of side effects linked to synthetic drugs has sparked a considerable interest in

natural products, resulting in an increase in research focused on their potential benefits [37-39]. While our previous research focused on the proximate composition, phytochemicals, and bioactive components of *Datura alba ness* (DAN), the current study aims to evaluate the potential analgesic activity of DAN extracts using preemptive and therapeutic analgesia models in Wistar rats, along with investigating the possible mechanism of action through molecular docking.

Our findings show that the methanolic leaf extract of *Datura alba ness* (MEDAN) significantly increased the pain threshold in male Wistar rats after three weeks of administration in a dose-dependent manner, with the 1000 mg/kg dose showing sustained effects. Combining MEDAN with Flexicam enhanced pain relief at higher doses (Table 1). However, for the therapeutic analgesic test, we observed that MEDAN increases pain thresholds within one hour but diminishes over time, with effects varying by dose and combination with standard drugs (Table 2). These observed analgesic effects of MEDAN could be attributed to a variety of bioactive compounds present in MEDAN. Our earlier finding showed the presence of some fatty acids (n-Hexadecanoic acid, Octadecanoic acid and Ricinoleic acid) plant sterols (Stigmasterol and Gamma-Sitosterol), Vitamins (Delta-Tocopherol (Vitamin E and [40]) and Triterpene (Squalene) [25] which have been shown to possess potent modulators of pain and inflammatory response [41-45]. These compounds have been reported to suppress pain and inflammation by inhibiting inflammatory cytokines which can contribute to pain relief or by inhibiting prostaglandin synthesis, which is involved in pain signaling. They have also been shown to be potent antioxidants which help in pain management by reducing oxidative stress and inflammation[41-45]. These findings indicate that *Datura alba ness* may possess analgesic properties, which could be amplified when combined with standard pain relief medications.

The present study also reveals that *Datura alba ness* significantly lowered serum C-reactive protein levels after three weeks of oral administration, both at all doses and in combination with a standard drug, compared to the standard drug, but showed no difference from the negative control.C-reactive protein (CRP) is an acute-phase protein synthesized by the liver in response to inflammation and stimulated by inflammatory cytokines, particularly interleukin-6 (IL-6)[46, 47]. They are often elevated in conditions associated with pain, such as arthritis, fibromyalgia, and other inflammatory diseases. The presence of CRP reflects ongoing inflammation, which is a key driver of pain[48, 49].Measuring CRP levels can help assess the severity of inflammation and, indirectly, the intensity of pain. Higher CRP levels often correlate with increased pain levels in various conditions[50]. Also, the molecular docking results from this study indicated that certain compounds in the extract, including stigmasterol, gamma-sitosterol, and cholest-5-en-3-ol, 24-propylidene-(3β), showed binding affinity to C-reactive protein receptors, mimicking the effects of the reference drug, celecoxib. Celecoxib is prescribed for symptomatic relief of osteoarthritisand rheumatoid arthritis in adults, as well as for acute pain from various causes. This medication acts as a selective noncompetitive inhibitor of the cyclooxygenase-2 (COX-2)

enzyme, which is predominantly expressed in inflamed tissues and is induced by inflammatory mediators [51]. Inhibiting COX-2 reduces the production of metabolites such as prostaglandin E2 (PGE2), prostacyclin (PGI2), thromboxane (TXA2), prostaglandin D2 (PGD2), and prostaglandin F2 (PGF2) [52]. The reduced level of serum CRP observed suggests a potent ability of MEDAN to reduce pain sensitivity and this maybe my potent than standard pain relief medication as observed in the study. These findings are in tandem with analgesic properties observed in other Datura species, *Daturafastuosa* and *Daturametel*[27, 28].

CONCLUSION

The use of natural products derived from medicinal plants as therapeutic remedies for pain management is gaining increasing attention due to their broad acceptability, availability, affordability, and minimal or nonexistent side effects. According to the data from the current study, methanol extracts from *Datura alba ness* leaves demonstrate significant efficacy in reducing pain by lowering C-reactive protein levels. This effect was particularly pronounced when the 500 mg/kg dose of MEDAN was combined with the standard drug, Flexicam. This study offers a scientific foundation that supports the traditional medicinal use of Datura alba Ness in treating pain and inflammation.

Ethical approval

The research design and protocol adhered to all guidelines regarding the use of laboratory animals. Ethical approval was obtained from the University of Port Harcourt Research Ethics Committee [REC/MM89/221].

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative Artificial intelligence (AI) technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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