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In vitro and animal model estimation of the anti-inflammatory and antinociceptive activities of *Abrus precatorius* (fabaceae) plant-mediated nanoparticles

Stephenie C ALARIBE [1*], Olayemi F ADEKOGBE [1], Blessing E TITILAYO [1], Felicia N EJIAH[2], Cynthia N NNADI [1], Sunday O OKOH [3], Mike O OJEMAYE [4], Oluwole B. FAMILONI [2], Anthony I OKOH [4]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, College of Medicine Campus, University of Lagos, Nigeria; [2]Department of Chemistry, University of Lagos, Akoka, Lagos, Nigeria; [3] CFET Department, Federal Institute of Industrial Research Oshodi, Lagos, Nigeria; [4] SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, South Africa

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Abstract

Background: Recent research indicates that biosynthesized metal nanoparticles from natural products such as plants often exhibit higher therapeutic efficacy and reduced toxicity compared to natural products at macro-scale or chemically-synthesized nanoparticles. The potential therapeutic compounds in natural products usually act as capping agents on synthesized nanoparticles, facilitating targeted delivery of those compounds at lower, more effective doses. Given the roles of inflammation, oxidative stress, and nociception in diseases, developing novel drugs with enhanced efficacy and reduced toxicity is crucial.

Objective: This study aimed to investigate the anti-inflammatory, antioxidant, and antinociceptive activities of *Abrus precatorius* extracts and their mediated nanoparticles using diverse *in vitro* and *in vivo* models.

Methods: The anti-inflammatory, antioxidant, and antinociceptive bioactivities of *Abrus precatorius* (AP) (Rosary pea) extracts were investigated. The extracts, labelled B2 for seeds, A2 for seed coats, and their mediated nanoparticles A1 (silver nanoparticles synthesized from seed coat extracts) and B1 (silver nanoparticles synthesized from seed extracts), were bio-synthesized using the crude extracts. Anti-inflammatory activity was assessed using the Carrageenan model and antinociceptive activity was evaluated through acetic acid writhing, hot plate, tail clip tests, and xylene ear oedema in mice, with doses ranging from 10-50 µg/kg. In vitro antioxidant activity was measured with 2,2-Diphenyl-1-picrylhydrazine (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays at concentrations of 10-50 µg/ml.

Results: The results showed that the aqueous extract of *Abrus precatorius* significantly reduced oedema volume in rats and increased antinociceptive and antioxidant activities, as did the mediated nanoparticle samples A1 and B1. In the acetic acid writhing test, the samples exhibited inhibition ranging from 5.5% to 83.3% at doses of 0.01-0.05 mg/kg, indicating reduced pain responses induced by acetic acid. Among the samples, B1 showed the highest inhibition in the acetic acid writhing test. In the DPPH scavenging radical test, sample B2 demonstrated the greatest inhibition of DPPH radicals at 88.64% at a concentration of 0.05 mg/ml, highlighting strong antioxidant activity. Sample A1 exhibited the highest inhibition at 80.42% for the carrageenan test after 6 hours, indicating potent anti-inflammatory effects.

Conclusion: The current research illustrates that the extract of *Abrus precatorius*, in both its crude and nanoparticle forms, displays notable anti-inflammatory, antioxidant, and antinociceptive properties. The biosynthesized nanoparticles prove to be more effective at lower doses in reducing oedema and scavenging DPPH radicals in rodents compared to the crude extracts.

Keywords: Anti-inflammatory, antioxidant, antinociceptive, nanoparticles, *Abrus precatorius*

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INTRODUCTION

Nanoparticles, according to the American Society for Testing and Materials (ASTM) standard definition, are particles with lengths ranging from 1 to 100 nanometers in two or three dimensions [1]. They are the smallest units capable of exhibiting integrated properties and transport characteristics [2]. Metal nanoparticles, such as silver nanoparticles, are increasingly utilized across diverse sectors including medicine, food, healthcare, consumer goods, and industry due to their unique physical and chemical attributes [3]. These nanoscale metallic particles exhibit distinctive properties that can significantly

* *Corresponding author*
Email: salaribe@unilag.edu.ng

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alter their physical, chemical, and biological behaviours, primarily due to their high surface-to-volume ratio [4]. Besides enhancing the bioavailability of therapeutic agents upon systemic or local administration in nanoparticle form, their physicochemical characteristics influence cellular uptake, distribution in the body, penetration through biological barriers, and subsequent therapeutic outcomes.

Inflammation is a vital biological response initiated by the body's immune system in reaction to harmful stimuli, including pathogens, damaged cells, or irritants. It serves a crucial role in immune defence by eliminating harmful substances and promoting tissue healing [5]. Persistent inflammation, characterized by sustained immune cell activation and the secretion of inflammation-promoting substances, can lead to tissue damage, impaired organ function, and systemic complications, contributing to the development of autoimmune disorders, cardiovascular diseases, neurodegenerative diseases, and cancer [6]. In contrast, nociception refers to the physiological mechanism of perceiving harmful stimuli and transmitting signals to the brain for analysis. While essential for survival as a warning system to avoid harm, abnormal nociceptive signalling can lead to long-lasting pain, nerve-related disorders, inflammation, heightened central nervous system sensitivity, and psychological consequences [7]. Chronic pain conditions, such as neuropathic and inflammatory pain, are prevalent and challenging to manage [8].

Although nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are fundamental pharmacological interventions for inflammation and pain, their long-term use is associated with adverse effects, including gastrointestinal complications, cardiovascular risks, tolerance, and addiction development. Moreover, the limited efficacy of existing therapies in certain patient populations underscores the urgent need for alternative treatment strategies [9,10].
Natural products, derived from plants, animals, or microorganisms, offer a diverse array of bioactive compounds with therapeutic potential. These compounds often possess unique chemical structures and mechanisms of action thus, making them valuable candidates for drug discovery and development [11]. Alternative therapies, such as those utilizing plant-mediated nanoparticles, hold promise for targeted delivery of these bioactive compounds, reduced systemic toxicity, and enhanced efficacy in alleviating inflammation and pain [12]. Leveraging the pharmacological properties of natural chemicals obtained from plants could overcome the limitations of current treatments, leading to safer, more effective, and personalized therapeutic interventions.

Abrus precatorius, commonly known as the rosary pea or jequirity, is a herbaceous flowering plant in the legume family Fabaceae. Despite its toxicity, it has a rich history of traditional medicinal use in Nigeria and other places in Africa for various conditions. For instance, the leaves and roots of this plant are used in South Africa to treat tuberculosis, bronchitis, whooping cough, chest complaints, and asthma [13]. In Nigeria, the leaves and other parts of the plant are used to treat cough, skin diseases, diarrhoea, and benign prostatic hyperplasia [14-16]. Different parts of the plant are used to treat asthma, sexual dysfunction, diabetes, cough, fever, sexually transmitted diseases, schistosomiasis, and gastropathology in Tanzania and Ghana [17].

Abrus precatorius contains diverse bioactive compounds, including flavonoids, alkaloids, and saponins, which contribute to its pharmacological activities [14,15]. Studies have demonstrated its anti-inflammatory, analgesic, antimicrobial, antioxidant, and immunomodulatory properties thus, highlighting its potential therapeutic applications. It is a unique source of many potential phytochemicals responsible for both its numerous therapeutic activities and its toxicity, with varying amounts present in different parts of the plant. Currently, over 166 compounds have been isolated from different parts of the plant [14]. This present study investigated the anti-inflammatory and antinociceptive activities of silver nanoparticles mediated by *Abrus precatorius*, employing both *in vitro* methods and *in vivo* models with adult albino Wistar rats and mice.

MATERIALS AND METHODS

Collection and extraction of *Abrus precatorius* (ap) plant specimens

Seeds of *Abrus precatorius* plants were collected from the Iseyin area, Oyo, south-west Nigeria, authenticated by a taxonomist, and deposited in the University of Lagos herbarium (ULH) with specimen voucher numbers ULH 2009b. The seed coats were separated, winnowed, and crushed from the seeds by mechanical aids. About 500 g of each sample was macerated in 2 L of distilled water and placed in a shaker for 24 hours. The mixture was filtered using filter paper. The filtrate was concentrated by lyophilization, and the extract was stored at -20°C for later use.

Synthesis and characterization of Ag nanoparticles with *Abrus precatorius* (Ap) extracts

The protocol used by Larayetan et al. [18] for the synthesis of silver nanoparticles (NPs) was adopted for this study. Briefly, one part of the aqueous extract of the plant part was added to nine parts of 0.10 M solution of silver nitrate. The mixture was then left to stir on a magnetic hot plate stirrer at temperature of 34°C ± 2°C until the visible colour change and precipitate noticed therein. The precipitate was thereafter centrifuged and washed in deionized water to attain clean nanoparticles. The product was then dried in an oven for 24 hours at 105 °C. The biosynthesized NPs were kept in a sealed sample amber bottle awaiting characterization and bioactive assays. The synthesized nanoparticle samples and the plant extract absorption spectra were examined with a Perkin-Elmer UV-visible absorption spectrophotometer. A Perkin-Elmer ATR 100 FTIR spectrophotometer was used to obtain the vibrational

frequencies of the Ag-Nano samples and the crude plant extract. The samples' transmission electron microscope (TEM) was produced at 100 kV (acceleration voltage) using the JOEL 1210 transmission electron microscope, while the electron micrograph was obtained via the JSM-6390 LVSEM scanning electron microscope (SEM) for the synthesized samples and seed aqueous extract. We also used SEM to amass the EDS (Energy Dispersive X-ray Spectroscopy) of the samples. The Bruker D8 X-ray diffractometer was utilized to confirm and measure the size, phase, and crystallinity of the nanomaterials. The samples were labelled as A1 (Ag-Nano of *Abrus precatorius* seed coat), A2 (crude seed coat of *Abrus precatorius*), B1 (Ag-Nano of *Abrus precatorius* seed), and B2 (crude seed of *Abrus precatorius*) and then stored in airtight containers.

In vivo assessment of anti-inflammatory and antinociceptive activities in albino Wistar rats and mice

The anti-inflammatory and antinociceptive activities of *Abrus precatorius* (AP) were investigated using an in vivo model with albino Wistar rats (111–149 g) and mice (18–29 g). The mice were procured from the animal laboratory of the College of Medicine, University of Lagos. They were adequately fed with mash (Top feed, Super Deluxe, Animal feed, manufactured by Premier Feed Mills Co. Ltd, Nigeria) and water and were kept and handled ethically in conformity with the Organization for Economic Cooperation and Development Guidelines (OECD) for the Testing of Chemicals (Section 4, Health Effects, Test No. 474, revised 2016) and under the international guiding principles for biomedical research involving animal. The enclosure maintained a 12-hour light/ dark cycle. The mice were initially weighed, and their average weights were used to prepare drug concentrations based on their doses. The activities were investigated to evaluate the samples' potential anti-inflammatory, analgesic, and pain-modulating properties.

Dosage Selection

The doses were chosen based on a previous study examining the subacute toxicity of intraperitoneal administration of *Abrus precatorius* seed, seed coat, and their nanoparticles in a mouse model [19]. The study concluded that a dose lower than 0.07 mg/kg of both biosynthesized and crude samples of *Abrus precatorius* is expected to be much safer in repeated-dose studies for assessment of therapeutic effects. Therefore, 0.05 mg/kg was selected as the highest dose and the mid-dose and lowest doses (0.02 mg/kg and 0.01 mg/kg respectively) differed with a factor of less than the root of 10 according to OECD guidelines for testing chemicals [20].

Carrageenan-induced oedema on the paws of rats

To evaluate the anti-inflammatory and anti-nociceptive activity of test drugs, two models were employed: the carrageenan-induced rat paw oedema test and the xylene-induced ear oedema test. The carrageenan-induced paw oedema test, a well-known model of acute inflammation widely used for screening novel anti-inflammatory compounds, was injected into the sub-plantar surface of the rat paw to induce biphasic oedema according to Shaban et al. [17], with modifications. Rats were divided into eleven groups of three in each group and fasted overnight before oral administration of distilled water (negative control), AP, and mediated nanoparticle extracts (0.01, 0.02, and 0.05 mg/kg), aspirin (10 mg/kg), and celecoxib (5 mg/kg) serving as reference standards. After 1 hour, 0.1 ml of 0.1% w/v carrageenan was injected into the sub-plantar right hind paw of rats to induce inflammation. Measurement (C_0) was made before the administration of the phlogistic agent (carrageenan). The extent of oedema was monitored at 1-hour intervals for 6 hours and determined by measuring the rat paw circumference (Ct) with a vernier caliper. The percentage inhibition was calculated as follows:

% Inhibition = 1– [D (test) ÷ D (negative control)] x 100

Where D = $Ct - C_0$

Where D is percentage inhibition

D (test) is difference in paw circumference in the drug-treated group.

D (negative control) is difference in paw circumference in the negative control group.

Hematological assay in carrageenan-induced oedema evaluation

About 1 ml of blood was obtained from the rats' retro-orbital sinus vein, and about 13 µl of whole blood volume was prepared for the Mindray analyzer. This was done by diluting about 0.1 ml of the blood from the test samples and control group into separate vials. A lysing agent, Lyse, was applied to the solution to ensure the cell membrane released its content. The assay was done to obtain and observe the white blood cell parameters.

Anti-nociceptive activities of *Abrus precatorius* crude and mediated ag-nanoparticles samples

Xylene-induced ear oedema test on mice ear

In the xylene-induced ear oedema test, xylene acts as a phlogistic (inflammatory) agent by increasing vascular permeability and leading to oedema formation characteristic of acute inflammation. Xylene-induced oedema partially involves substance P, a neurotransmitter in the central nervous system, which induces nitric oxide, resulting in vasodilation and plasma exudation and an inflammatory response of post-capillary venules [19]. Mice were divided into five groups of both sexes and fed adequately with mash and water. They were fasted in an enclosure where a 12-hour light/ dark cycle was maintained before the administration of the test agents. Nanophilized AP and extracts were administered orally at different doses (50, 20, and 10 µg/kg) in the treatment groups for four consecutive days. One (1) hour after the last dose, 30 uL of xylene was smeared on the surface of the left ear, with the right ear serving as a control for each animal in all the groups.

The animals were sacrificed under anesthesia (Ketamine (0.5 mL) was used and followed by cervical dislocation to ensure that the animals' death is painless as well as humane

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according to International guidelines (OECD)) and the auricles were cut off according to Pan et al. [22]. The auricle diameter was measured and the masses were weighed to ascertain the difference between the two ears. 0.1% normal saline was used as a negative control and 2 mg/kg of aspirin as a positive control. The inhibition rate of the control and treatment groups was calculated as follows:

% Inhibition = E(control) - E(treatment) / E ¬ (control) x 100
Degree of oedema (E) = Mright – Mleft
Where M is the weight of the mouse ears.

Acetic acid writhing test

Ten groups of mice that fasted for 12 - hours were employed for the study. The positive control groups were treated with aspirin and co-codamol (5 mg/kg each) before nociception. The negative control group was treated with only distilled water. The number of writhes recorded as abdominal contractions and limb extension were taken in a time window of 30 minutes over an interval of 5 minutes, according to Mondal et al. [23]. A single intraperitoneal injection (10 mL/kg) of 0.6% of acetic acid was administered orally through a feeding tube after 60 minutes. A preliminary screening was carried out on a group to determine an estimated reaction time. The following treatment was then administered: normal saline (10 mL/kg), *Abrus precatorius* (0.01 mg/kg, 0.02 mg/kg, and 0.05 mg/kg), and acetylsalicylic acid (5 mg/kg). The reaction time of each mouse to the nociception was determined route-dependently, according to Ferreira et al. [24].

Hot plate test

Ten groups of mice were employed to study qualitatively the effects of heat on the mice tissues. The reaction time, the mean, and the standard deviation were obtained. The hot plate employed had a heat-absorbent mantle, which helped in retaining heat. The hot plate had its heat turned to a third of its max heat, (50 – 60 ºC), then regulated to a lower heating temperature (15 ºC). The hot plate's mantle was supported by layers of cellulose paper to provide some level of insulation at the 50 – 55 ºC surface, which represents some variation according to Masocha et al. [25]. The treatment drugs and control: *Abrus precatorius* samples A1, B1, A2, and B2 were administered at three dose levels (0.01, 0.02, and 0.05 mg/kg), with co-codamol and acetylsalicylic acid (5 mg/kg) as positive controls and normal saline as the negative control. The antinociceptive activity of the mice on the hotplate was tested for possible inhibitory action. The inhibition percentage was calculated as follows:

% Percentage Inhibition = (Reaction time control – Reaction time standard or treatment) / Reaction time control

Tail clip test

Ten groups of mice were used for the study of the effects of *Abrus precatorius* samples on the reaction time of a mouse that has had its tail clamped, according to Singh [26]. The reaction time was calculated as a latency period (mean ± standard deviation), and the onset time was noted as mean ± standard deviation. Values less than an evaluated cut-off time are termed insignificant latencies.

Scavenging activities of AP-mediated nanoparticles and extracts - DPPH radical scavenging assay of AP-mediated nanoparticles and extracts

The scavenging activity of the extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined as described by Alaribe et al. [27], with slight modifications. A 0.01 M solution of DPPH was diluted to make four concentrations. An aliquot of 0.25 ml of aqueous extract at different concentrations (0.010, 0.020, and 0.050 mg/mL) was mixed with 1 ml of 0.1 mM DPPH solution.

The mixture was vigorously shaken and left to stand at room temperature for 30 minutes. Ascorbic acid was used as a positive control. All readings were taken in duplicate. After 30 minutes, the absorbance of each mixture was measured at a wavelength of 517 nm. The radical scavenging effect was calculated using the equation below:

DPPH scavenging effect (%) = (A0 - A1)/A0 × 100

Where A0 is the absorption of the blank solution and A1 is the absorption of the extract or ascorbic acid solution.

Ferric reducing antioxidant power (FRAP) capacity assay

The ferric-reducing antioxidant, according to Zhong and Shahidi; Benzie and Devaki [28,29], which uses the measurement of a ferric-ferrous complex absorbance as a metric for assessing the antioxidant capacity of a sample with ascorbic acid as a reference standard, was employed for this study. The FRAP reagent was prepared by mixing 0.5 mL of the 0.2 M phosphate buffer (pH 7.0), 1% w/v K_3Fe (CN) 6 solution, and 0.25 mL of each test sample solution. The resulting mixture was incubated for 20 minutes at 50 ºC. Then 0.5 mL of 10% trichloroacetic acid was added. The mixture was centrifuged in sample bottles at speeds of 3000 rpm for 10 minutes. About 1.4 mL of distilled water was added to the top layer following the addition of 0.3 mL of 4.5 mg/mL $FeCl_3$ solution, after which a blue complex formed on it. The absorbance was taken using a double-beam UV spectrophotometer at a wavelength of 700 nm. The concentration of the reference standard ranged from 0.0034 – 0.0068 mg/mL, and the concentration of the samples ranged from 0.005 – 0.05 mg/mL.

Data Analysis

Statistical analysis was performed using GraphPad Prism 9.5 (GraphPad Software Inc.; San Diego, CA, USA). The difference across multiple groups was compared with ANOVA test. Tukey's post hoc multiple comparison test was carried out to determine significance relative to the control groups.

RESULTS

The results from acute inflammatory studies using different animal groups - A1DI (0.01 mg/kg), A1DII (0.02 mg/kg),

A1DIII (0.05 mg/kg), A2DI (0.01 mg/kg), A2DII (0.02 mg/kg), A2DIII (0.05 mg/kg), Control, Celebrex, and ASP (Aspirin, 10 mg/kg) - through the carrageenan-induced Inflammation method, showed the percentage inhibition of inflammation for carrageenan-induced rat paw oedema test after the 6th hour (Table 1). A common trend in the degree of oedema and percentage inhibition was observed as follows: A1 groups assumed a perfectly linear trend while the A2 groups did not. Inhibition percentages were highest at A1DIII for A1 and A2DI for A2.

A1DIII had a higher inhibition than the aspirin control group; unexpected negative inhibition values recorded in the B1 groups are consequences of single-dose administration, which might imply a disparity between the effective dose and other implications. The results are presented in Figure 1(A), showing the comparative inhibition between A1 and A2 samples in the carrageenan assay after 6 hours, and Figure 1(B): Comparative inhibition for B1 and B2 after 6 hours. Figure 1(E) shows the trendlines of percentage inhibition for all (A1/A2) treatment groups plotted against time (after 6 hours) in the carrageenan test. A composite column bar chart, Figure 1 (D), is used to compare and contrast the performance of the B1 and B2 groups.

In Figure 1(A), the trendlines shown as lines-of-best-fit show a bit of contrast between groups A1 and A2. In Figure 1(B), B2 at 0.05 concentration recorded good inhibition, seen as the orange bar. In Figure 1(C), a strong correlation is observed between the degree of oedema and the % inhibitions recorded for A1 and A2, as estimated from their respective lines of best fit. Significantly, A1DIII had the highest inhibition in reducing the oedematous action of carrageenan. Table 2 shows the degree of oedema and percentage inhibition of inflammation per group for the xylene ear oedema test for the different groups; A1DI (sample A1 - 0.01 mg/kg), A1DII (sample A1 - 0.02 mg/kg), A1DIII (sample A1 - 0.05 mg/kg), B2DIII (sample B2 - 0.05 mg/kg), B1DIII (sample B1 - 0.05 mg/kg), NS 0.1% (0.1 % normal saline). Figure 3 (A) shows that A2DI inhibited the oedema formation on the auricle. It exhibited a wide confidence interval compared to its true value. A2DII, A1DI, and A1DIII also inhibited xylene phlogistic action. The seed coat samples inhibited inflammation at their lower concentrations.

Figures 3(B) and 3(C) show trends in the number of writhes against dose. Writhing, among other reactions, was observed during the acetic acid test. The number of writhes appeared to have a direct relationship with the doses for the A-treatment group. Figures 3(A) and 3(B) depict trends in the number of writhes against dose. Writhing, among other reactions, was observed during the acetic acid test. The number of writhes appeared to have a direct relationship with the doses for the A-treatment group. The number of writhes seemingly increased as the doses for the A-treatment group increased, which underscores poor inhibition for the A1 and A2 treatment groups. Conversely, this was not observed for the B1 and B2 groups. Specifically, B1 at 0.05 mg/kg (B1DIII) exhibited the greatest inhibition for the Acetic Acid Writhing test. Figures 3(D) and (E) show trends in the inhibition percentage against dose. In Figure 3(F), the comparative inhibition of A1 and A2 is shown, while Figure 3(H) displays the comparison of B1 and B2 in the mouse tail clip test (TCT). The range of inhibition in the TCT for Abrus precatorius samples ranged from 4.12 to 36%, with the highest inhibition observed in the A1DII group, closely followed by A2DI.

Figure 3(G) shows a dual plot of comparative latency times (gray vs sunset yellow trendlines) and onset times (blue vs orange trendlines) against dose between samples A1/A2 in the tail clip test. The nociception at the tail was assessed by obtaining a cut-off time of 5.10 seconds, where times higher than the cut-off are considered a positive response, indicating analgesic and/or antinociceptive activity. Figure 3 – (I) illustrates the comparative reaction times for samples A1 and A2 in the hot plate test, showing a linear dependence on dose for both samples. Sample A2 generally recorded higher reaction times compared to A1. Additionally, Figure 3 – (K) displays the comparative inhibition for B1 and B2 in the Hot Plate Test. The range of percentage inhibition for the Abrus precatorius samples ranged from (-2.88) to (-78.5), with none of the samples inhibiting or aiding the response to heat felt at the foot paw of the mice. Antioxidant results are as presented in Table 3, for 2,2-Diphenyl-1- Picrylhydrazine Radical (DPPH) Ultraviolet scavenging assay and Ferric Reducing Antioxidant Power Assay (FRAP).

Table1. The percentage inhibition of inflammation per group for carrageenan-induced rat paw oedema after the 6th hour

Treatment Group	DOSE (mg/kg)	Ct – C0 (cm) (Degree of oedema after 6h)	% Inhibition
Control	10	1.89 ± 0.30	--
$A1D^{I}$	0.01	1.39 ± 0.40	$26.5 \pm 0.28^{a,b}$
$A1D^{II}$	0.02	0.79 ± 0.23	$58.2 \pm 0.22^{a,b}$
$A1D^{III}$	0.05	0.36 ± 0.48	$80.5 \pm 0.33^{a,b}$
$A2D^{I}$	0.01	0.91 ± 0.54	$51.9 \pm 0.36^{a,b}$
$A2D^{II}$	0.02	1.15 ± 0.49	$39.2 \pm 0.33^{a,b}$
$A2D^{III}$	0.05	1.14 ± 0.10	$39.7 \pm 0.18^{a,b}$
$B1D^{III}$	0.05	1.98 ± 0.80	$-4.76 \pm 0.49^{a,b}$
$B2D^{III}$	0.05	0.31 ± 0.29	60.32 ± 0.24^{b}
ASP	10	0.72 ± 0.03	61.9 ± 0.17
CELEBREX (CEL)	5	1.73 ± 0.76	8.47 ± 0.47

Significant differences from ASP group ($p<0.05$)
b: Significant difference from Celebrex group ($p<0.05$)
A1DI (0.01mg/kg), A1DII (0.02mg/kg), A1DIII (0.05mg/kg), A2DI (0.01mg/kg), A2DII(0.02mg/kg), A2DIII (0.05mg/kg), B1DIII (0.05mg/kg), B2DIII(0.05mg/kg), ASP (Aspirin) and Celebrex

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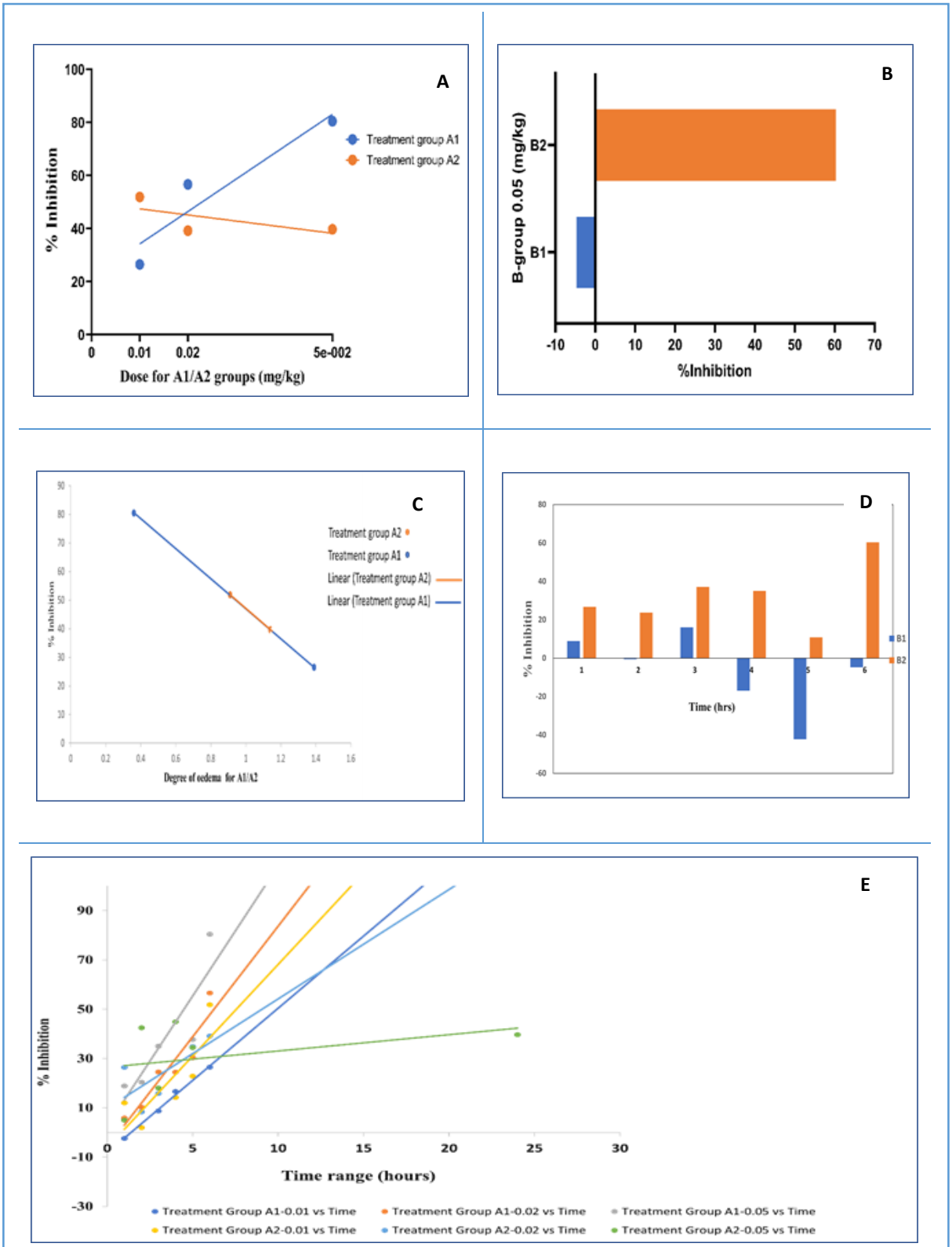

Figure 1. Results of the anti-inflammatory assay showing the comparative inhibition percentage of groups A1 and A2 after 6 hours (A), comparative inhibition percentage of groups B1 and B2 after 6 hours (B), comparative inhibition of groups A1 and A2 in the carrageenan assay after 6 hours (C), comparative inhibition for B1 and B2 against time (D), and comparative inhibition for groups A1 and A2 against time (E).

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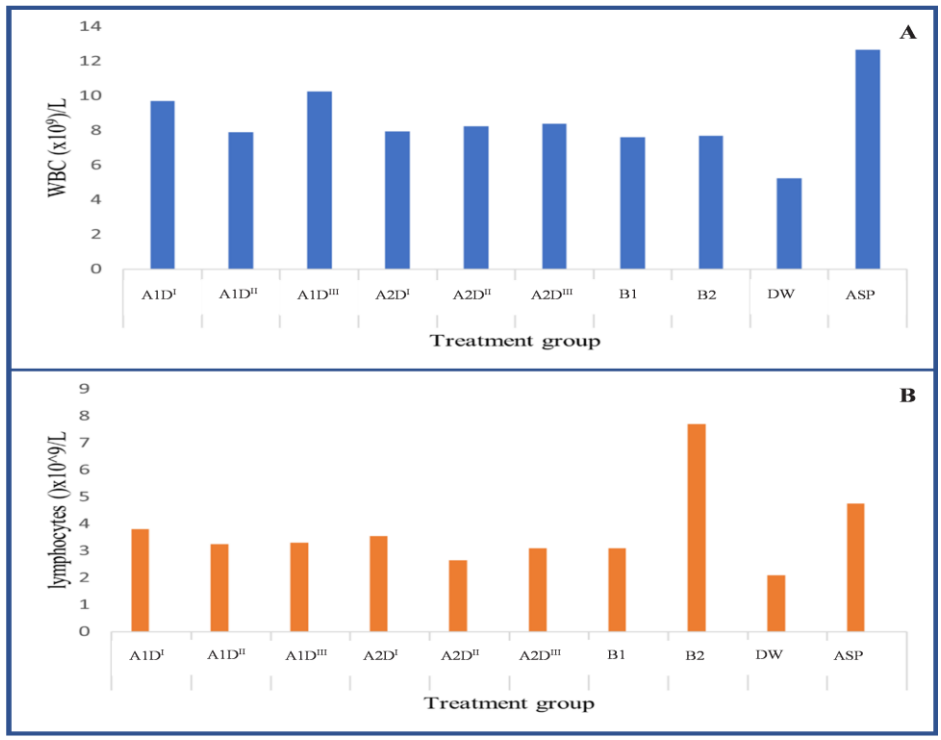

Figure 2. Results of the haematological assay showing the white blood cell count per treatment group (A), and comparative lymphocytes count per treatment group (B).

Table 2. The degree of Oedema and percentage inhibition of inflammation per treatment group for xylene ear oedema test

TREATMENT	LEFT EAR	RIGHT EAR	DEGREE OF OEDEMA	% INHIBITION
$A1D^{I}$	0.0676±0.006	0.0764±0.0187	0.0088±0.0131	43.59
$A1D^{II}$	0.0575±0.007	0.0683±0.0063	0.0108±0.0008	30.76
$A1D^{III}$	0.0494±0.002	0.0743±0.0084	0.0249±0.0061	-54.96
$A2D^{I}$	0.0576±0.007	0.0628±0.0161	0.0052±0.0093	66.67
$A2D^{II}$	0.0524±0.004	0.0641±0.0067	0.0117±0.0031	25.00
$A2D^{III}$	0.0612±0.007	0.0930±0.0088	0.0318±0.0021	-103.8
$B1D^{III}$	0.0628±0.005	0.0865±0.0272	0.0237±0.0225	-51.92
$B2D^{III}$	0.0547±0.004	0.0820±0.0094	0.0273±0.0197	-75
NS 0.1%	0.0577±0.004	0.0733±0.0232	0.0156±0.0193	N/A
INDOMETHACIN	0.0611±0.005	0.0735±0.0111	0.0124±0.0064	20.51

Table 3. The comparative Ferric reducing Antioxidant Assay (FRAP) and percentage inhibition of DPPH radical scavenging during Ultraviolet monitoring for all groups.

	FRAP Values				Percentage Inhibition %			
Concentration (mg/ml) Groups	A1	A2	B1	B2	A1	A2	B1	B2
0.005	6.36	5.91	5.94	6.91	-41.58	-1.84	-5.26	-36.32
0.01	4.27	4.06	4.65	4.48	40.85	44.79	49.15	25.21
0.02	3.34	3.39	3.47	3.83	69.14	70.58	64.75	65.32
0.05	2.74	2.77	2.97	2.91	85.95	88.40	83.96	88.64

* Comparative inhibition between the treatment groups.

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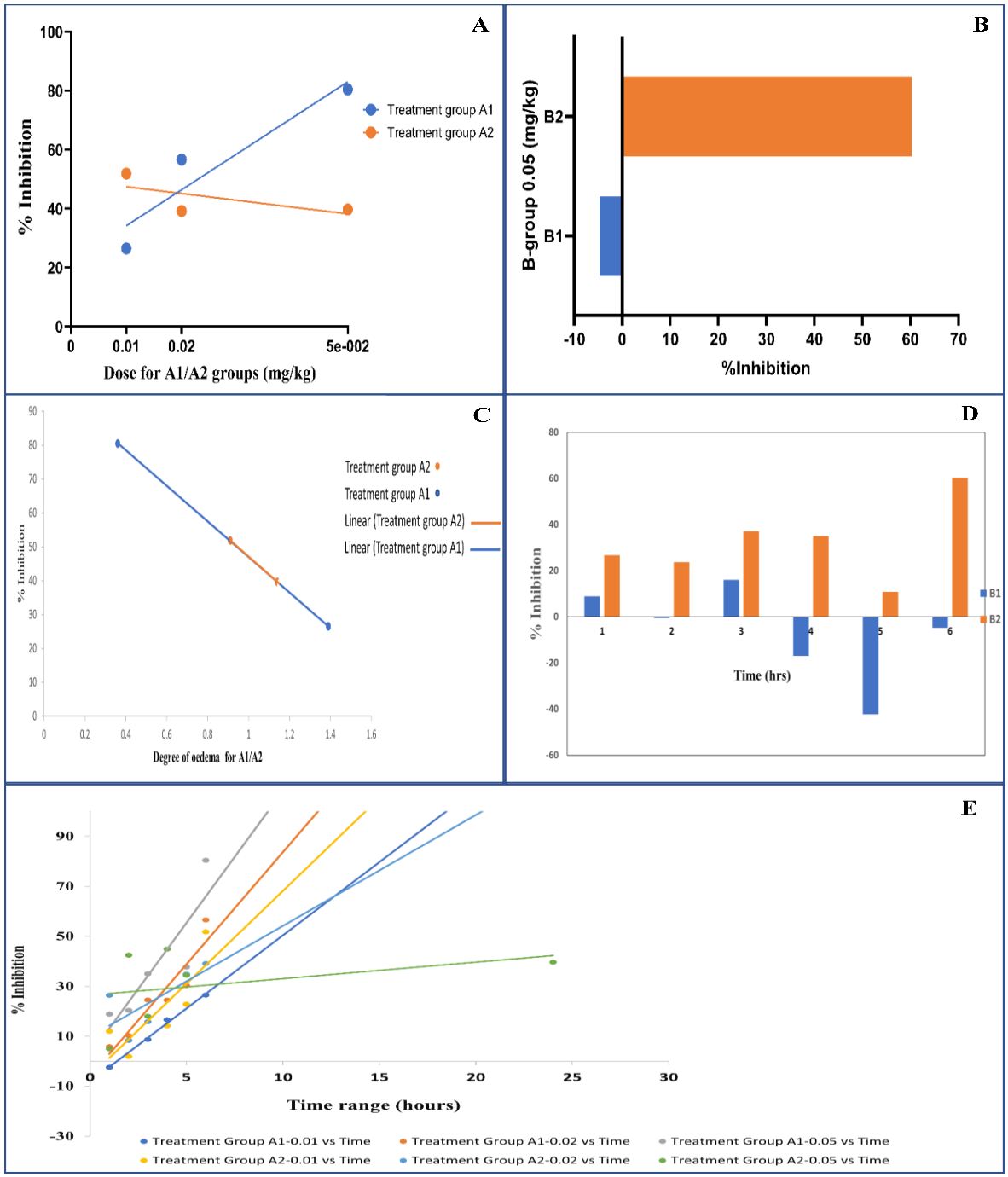

Figure 3. Results of the anti-inflammatory assay showing the degree of oedema in the xylene ear oedema test (A), acetic acid writhes for treatment groups A1 and A2 (B), acetic acid writhes for treatment groups B1 and B2 (C), comparative inhibition for A1 and A2 through the acetic acid writhing test (D), comparative inhibition for B1 and B2 treatment groups in the acetic acid writhing test (E), comparative inhibition for A1 and A2 treatment groups in the Tail Clip Test (F), comparative latency/onset time for A1 and A2 treatment groups in the Tail Clip Test (G), comparative inhibition for B2 and B1 treatment groups in the Tail Clip Test (H), comparative reaction time for A2 and A1 treatment groups in the Hot Plate Test (I), comparative inhibition for A1 and A2 in the Hot Plate Test (J), and comparative inhibition for groups B1 and B2 in the Hot Plate Test (K).

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DISCUSSION

Inflammation is a major hallmark of various chronic conditions, such as cancer, arthritis, cardiovascular disease, and diabetes. *Helicobacter pylori* infection in ulcer patients, for example, may be linked with inflammation [30]. Various anti-inflammatory medications have been developed, but current drug therapy is associated with serious and deleterious long-term side effects such as gastrointestinal irritation, bronchospasm, increased risk of stroke and heart attacks, fluid retention, kidney failure, and prolonged bleeding time [31]. To minimize the potential risk of an adverse event, numerous regulatory bodies and medical practitioners recommend using the lowest effective NSAID dose for the shortest time necessary, consistent with individual patient treatment goals [32]. Low-dose NSAIDs could minimize the side effects of these drugs while maintaining their clinical efficacy and effectiveness.

A novel approach to improving safety through lower dosing involves the application of as nanotechnology, which involves production of drug particles approximately smaller than conventional drug particles. The decreased particle size increases the total surface area of the particle, which allows for faster dissolution of the drug. Thus, nanotechnology may enable drugs to be delivered to ecise anatomical sites in the body and to release drug doses on a predetermined schedule for optimal treatment [33]. This offers improved efficacy of treatment and reduction of the toxicity that accompanies narrow therapeutic index and high doses. Hence, there are potential benefits associated with new low-dose nanoparticulate formulations for the treatment of inflammation, which are explored in this study.

This study also explored the potential of herbal medicine in the advancement of therapeutics through nanotechnology, by investigating the use of *Abrus precatorius* as an anti-inflammatory agent. Experiments were carried out at therapeutic doses, below the LD50 determined to be 0.71 mg/kg on the in vivo evaluation of the subacute toxicity of biosynthesized silver nanoparticles from *A. precatorius* on the cells of adult albino mice. The bioactive compounds present in *Abrus precatorius* L. play a vital role in reducing and stabilizing silver ions present in silver nitrate solution, leading to the formation of AgNPs with potential biomedical applications. From the results of the Carrageenan-induced rat paw oedema, the early phase observed at approximately 1 hr is related to the release of histamine, serotonin, bradykinin, and to a lesser extent, prostaglandins produced by cyclooxygenase enzymes, whereas the delayed phase (after 1 - hour) is attributed to neutrophil infiltration, and the continuing of prostaglandin generation. Release of the neutrophil-derived free radicals, nitric oxide (NO), and pro-inflammatory cytokines such as tumour necrosis factor (TNF-α) and interleukin-1 β (IL-1 β) are also known to be involved in the delayed phase of carrageenan-induced acute inflammation [34].

In the carrageenan-induced rat paw experiment, AP extracts exhibited desirable anti-inflammatory activity. This is consistent with the study of Omoboyowa [35], in which the ethyl acetate extract of *Abrus precatorius* seed demonstrated prophylactic anti-ulcerogenic activity in EtOH/HCl gastric injury-induced rats by restoring elevated stomach ulcerogenic scores which could be due to the down-regulation of pro-inflammatory genes (COX-2, INOS, IL-1β, NF-kβ, and TNF-α) that were observed to be up-regulated by the administration of acidified ethanol in rats. In this present study, there was a time-dependent increase in paw oedema following the administration of carrageenan, then a subsequent decrease after the first two hours. This was the case for most groups, except for the negative control, where the inflammatory response peaked at the third hour. Specimens treated with sample A1 (seed coat AP-AgNPs) showed a dose-dependent decrease in inflammation, A1DI (26.5%), A1DII (58.2%), and A1DIII (81.0%). At higher doses, A1 demonstrated a faster onset of action. When juxtaposing equivalent doses of AP seed coat nanoparticulate extract and its crude extract, AP nanoparticles proved to be more efficacious, with the exception of A1DI and A2DI; a low dose of the crude seed coat, A2DI showed higher inhibition (51.9%) compared to the nanoparticle A1DI (26.5%).

At the lowest dose, the crude seed coat extract (51.9% inhibition) yielded comparable inhibitory effects to aspirin (61.9% inhibition). Meanwhile, 0.05 mg/kg of the crude seed (B2DIII) exhibited the highest anti-inflammatory activity. Its nanoparticulate form had negative inhibition, indicating pro-inflammatory behaviour. While the exact mechanism of this event is not known, it may be attributed to the high dose; if the crude seed has been shown to be very potent, then its nanoparticulate form is expected to have better bioavailability, which may explain its probable toxicity at higher doses. Further study with lower doses may provide more insight into the therapeutic potential of AP seeds as nanoparticles and thus provide evidence for comparison to crude seeds. Overall, the carrageenan-induced rat paw experiment showed the *Abrus precatorius* nanoparticles to be equally effective anti-inflammatory agents as their crude forms.

The results obtained from the xylene-induced mice ear oedema test showed a sustainable disparity from the carrageenan test. The crude seed coat extract demonstrated highest anti-inflammatory activity at the lowest dose, with a percentage inhibition of 66.67. At the highest doses, all samples exhibited negative inhibition. This unusual pattern may be attributed to the low therapeutic index (0.5) of *A. precatorius* which indicates high toxicity of the plant. This may be due to the presence of the toxic component called abrin [36]. Increasing the dose of a drug with a narrow therapeutic index increases the probability of toxicity or ineffectiveness of the drug [37].

The anti-inflammatory findings in this study had similarly been demonstrated in the results of Owunari [38] in the leaf extract of the plant *A. precatorius*, which produced a significant reduction in the croton oil-treated rat ear (67.10

+ 2%), thus exhibiting potent anti-inflammatory activity less than that produced by acetylsalicylic acid (71.1 + 2%), a reference drug used in this study. Negative values observed during the experiment and data analysis may be due to the fact that the seeds are cytotoxic and might have some effect on their inhibitory actions through the regulation of cellular pathways. This might explain why the seeds are phytochemically active at lower concentrations compared to the 200 - 400 mg/kg doses common in the literature for ethanolic leaf extract. Results of statistical significance showed that acetic acid latency times were significantly related to the onset times of action in the dose classes for treatment groups. The one-way ANOVA and Tukey test showed that at $p < 0.01$ level of significance, the antinociception can function in two distinct stages for neurogenic pain and inflammatory pain. A good antinociceptive is expected to be active in the two stages.

Conclusion

The present study indicates that *Abrus precatorius* extract had anti-inflammatory activities. It reduced mice ear oedema and rat paw oedema in a dose-dependent manner. The carrageenan-induced rat paw oedema test proved the AP seed coat nanoparticulate extract to be effective as its crude form in treating inflammation. The nanoparticulate formulation did not show a significant advantage over the crude extract.

Further studies that address the aforementioned experimental inconsistencies can be carried out to provide more insight. The activities of *Abrus precatorius* showed that the nanoparticle samples showed better activity and were effective at low doses when administered to the rodents. They showed significant antioxidant scavenging against DPPH radicals and inhibited the actions of acetic acid in the abdomen of treated mice, and they did inhibit carrageenan-induced rat paw oedema. The results from the antioxidant, anti-inflammatory and antinociceptive activities studies suggest that the seed and nanoparticle seed samples (without seed coat) of *Abrus precatorius* had a greater inhibition of inflammation (carrageenan oedema), nociception, and oxidation (DPPH scavenging assay) when compared to the recorded inhibition percentages in previous studies.

DECLARATIONS

Ethical consideration

All animal experimental procedures were approved by the Health Research Ethics Committee of the College of Medicine of the University of Lagos with approval number CMUL/ACUREC/02/22/1043

Consent to publish

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding

None

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

SCA participated in conceptualization, design, and supervision. OFA, TEB, CNN, AIO, SOO, and MOO participated in the data acquisition. SCA, OFA, TEB, CNN participated in the manuscript writing. FNE and OBF reviewed the manuscript.

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Availability of data

Data is available upon request to the corresponding author.

REFERENCES

1. Kim, S., et al., Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicology in vitro, 2009. 23(6): p. 1076-1084.
2. Ghalanbor, Z., S.-A. Marashi, and B. Ranjbar, Nanotechnology helps medicine: nanoscale swimmers and their future applications. Medical hypotheses, 2005. 65(1): p. 198-199.
3. Wong, L., G. Chong, and J. Ordóñez. Biosynthesis of copper nanoparticles from leaching solutions obtained from mining tailings as raw material. in International Conference on Functional Nanomaterials and Nanodevices. Prague, In. 2019.
4. Cheng, W., et al., Bagasse-derived Carbon-supported Ru nanoparticles as Catalyst for Efficient Dehydrogenation of Ammonia Borane. ChemNanoMat, 2020. 6(8): p. 1251-1259.
5. Chen, L., et al., Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 2018. 9(6): p. 7204.
6. Furman, D., et al., Chronic inflammation in the etiology of disease across the life span. Nature Medicine, 2019. 25(12): p. 1822-1832.
7. Yang, S. and M.C. Chang, Chronic pain: structural and functional changes in brain structures and associated negative affective states. International journal of molecular sciences, 2019. 20(13): p. 3130.
8. Attal, N., D. Bouhassira, and L. Colvin, Advances and challenges in neuropathic pain: a narrative review and future directions. British Journal of Anaesthesia, 2023. 131(1): p. 79-92.
9. Rotunno, R., et al., NSAIDs and heart failure: A dangerous relationship. Monaldi Archives for Chest Disease, 2018. 88(2).
10. Domper Arnal, M.-J., G. Hijos-Mallada, and A. Lanas, Gastrointestinal and cardiovascular adverse events

associated with NSAIDs. Expert Opinion on Drug Safety, 2022. 21(3): p. 373-384.

11. Yuan, H., et al., The traditional medicine and modern medicine from natural products. Molecules, 2016. 21(5): p. 559.
12. Berta, L., et al., A review on plant-mediated synthesis of bimetallic nanoparticles, characterisation and their biological applications. Materials, 2021. 14(24): p. 7677.
13. Madikizela, B., M.A. Aderogba, and J. Van Staden, Isolation and characterization of antimicrobial constituents of Searsia chirindensis L.(Anacardiaceae) leaf extracts. Journal of ethnopharmacology, 2013. 150(2): p. 609-613.
14. Qian, H., et al., The traditional uses, phytochemistry and pharmacology of Abrus precatorius L.: a comprehensive review. Journal of Ethnopharmacology, 2022. 296: p. 115463.
15. Okoro, E. E., Ahmad, M. S., Osoniyi, O. R., & Onajobi, F. D. (2020). Antifungal and antileishmanial activities of fractions and isolated isoflavanquinones from the roots of Abrus precatorius. Comparative Clinical Pathology, 29(2), 391-396
16. Adelowotan, O., Aibinu, I., Adenipekun, E., & Odugbemi, T. (2008). The in-vitro antimicrobial activity of Abrus precatorius (L) fabaceae extract on some clinical pathogens. Niger Postgrad Med J, 15(1), 32-37.
17. Kuete, V., Toxicological survey of African medicinal plants. 2014
18. Larayetan, R., et al., Silver nanoparticles mediated by Callistemon citrinus extracts and their antimalaria, antitrypanosoma and antibacterial efficacy. Journal of Molecular Liquids, 2019. 273: p. 615-625.
19. Alaribe, S. C., Titilayo, B. E., Ejiah, F. N., Oloruntoba, V. A., Okoh, S. O., Osiagwu, D. D., Ojemaye, M. O., Familoni, O., & Okoh, A. I. Biosynthesized Silver Nanoparticles from Abrus Precatorius: Subacute Toxicity Assessment and Bioimaging Analysis for Enhanced Pharmacological Insight. FUW Trends in Science & Technology Journal. 2024. 9(2), 480-498
20. Chemicals, D. O. F. O., OECD Guideline for testing of chemicals. The Organisation for Economic Co-operation and Development: Paris, France, 2005: p. 1-13.
21. Singsai, K., et al., Antilipoxygenase and anti-inflammatory activities of Streblus asper leaf extract on xylene-induced ear edema in mice. Advances in pharmacological and pharmaceutical sciences, 2020. 2020.
22. Pan, L., et al., The treatment of cholecystitis and cholelithiasis by Tibetan medicine. Evidence-Based Complementary and Alternative Medicine, 2021. 2021.
23. Mondal, S., et al., Toxicological studies and assessment of pharmacological activities of Abrus precatorius L.(Fabaceae) ethanolic leaves extract in the management of pain, psychiatric and neurological conditions: An in-vivo study. Journal of Applied Pharmaceutical Science, 2017. 7(2): p. 207-216
24. Ferreira, M., et al., Analgesic and anti-inflammatory activities of a fraction rich in oncocalyxone A isolated from Auxemma oncocalyx. Phytomedicine, 2004. 11(4): p. 315-322.
25. Masocha, W., S.B. Kombian, and I.O. Edafiogho, Evaluation of the antinociceptive activities of enaminone compounds on the formalin and hot plate tests in mice. Scientific Reports, 2016. 6(1): p. 21582
26. Singh, l., A study of analgesic activity of haffner's tail clip method on albino wistar rats of some polyherbal formulation. Asian J Pharm Clin Res, 2019. 12(9): p. 142-144.
27. Alaribe, C.S., A.R. Oladipupo, and I.A. Ariyo, Total phenolic and flavonoid contents, metabolic profiling, and in vitro and in silico antioxidant studies of the leaf and stem extracts of Acalypha fimbriata Schum. & Thonn.(Euphorbiaceae). Ethiopian Pharmaceutical Journal, 2021. 37(1): p. 1-14.
28. Zhong, Y. and F. Shahidi, Methods for the assessment of antioxidant activity in foods, in Handbook of antioxidants for food preservation. 2015, Elsevier. p. 287-333.
29. Benzie, I.F. and M. Devaki, The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. Measurement of antioxidant activity & capacity: Recent trends and applications, 2018: p. 77-106.
30. Alaribe, C., et al., Cytoprotective, healing and anti-Helicobacter pylori activities of stem bark powder extracts and butanol fraction (BNL) of alkaloid rich Nauclea latifolia SM (Rubiaceae). 2018.
31. Ghlichloo, I. and V. Gerriets, Nonsteroidal anti-inflammatory drugs (NSAIDs). 2019.
32. Matthews, M.L., The role of dose reduction with NSAID use. The American Journal of Managed Care, 2013. 19(14 Suppl): p. s273-7.
33. Patra, J.K., et al., Nano based drug delivery systems: recent developments and future prospects. Journal of nanobiotechnology, 2018. 16: p. 1-33.
34. Mansouri, M.T., et al., A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian journal of pharmacology, 2015. 47(3): p. 292-298.
35. Omoboyowa, D.A., et al., Protective potential of ethylacetate extract of Abrus precatorius (Linn) seeds against HCl/EtOH-induced gastric ulcer via pro-inflammatory regulation: In vivo and in silico study. Phytomedicine Plus, 2021. 1(4): p. 100145.
36. Saganuwan, S.A., Comparative therapeutic index, lethal time and safety margin of various toxicants and snake antivenoms using newly derived and old formulas. BMC Research Notes, 2020. 13: p. 1-7.
37. Abimbola, F. Dose-Response Relationships – Clinical Pharmacology. 2022; Available from: https://www.merckmanuals.com/professional/clinical-pharmacology/pharmacodynamics/dose-response-relationships.
38. Georgewill, O.A. and U.O. Georgewill, Evaluation of the anti-inflammatory activity of extract of Abrus precatorious. Eastern Journal of Medicine, 2009. 14(1): p. 23.