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Noscapine shows antimalarial activity against *Plasmodium falciparum* 3D7, its clinical isolate Pf140/SS, and *Plasmodium berghei* ANKA

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ABSTRACT

Objective: To evaluate the antimalarial activity of noscapine against *Plasmodium falciparum* 3D7 strain (Pf3D7), its clinical isolate (Pf140/SS), and *Plasmodium berghei* ANKA (PbA).

Methods: Using ring-stage survival assay, phenotypic assessments, and SYBR-green-based fluorescence assay, the antimalarial activities of noscapine were assessed compared with dihydroartemisinin (DHA) in *in vivo* and *in vitro* studies. In addition, the hemolysis and cytotoxicity tests were carried out to evaluate its safety. PCR assay was also conducted to determine the effect of noscapine on the papain-like cysteine protease of *Plasmodium falciparum* falcipain-2 (PfFP-2).

Results: The antimalarial efficacy of noscapine against Pf3D7 and Pf140/SS was comparable to DHA, with IC_{50} values of (7.68±0.88) and (5.57±0.74) nM/mL, respectively, and >95% inhibition of PbA infected rats. Noscapine also showed a safe profile, as evidenced by low hemolysis and cytotoxicity even at high concentrations. Moreover, *PfFP–2* gene expression was significantly inhibited in both noscapine-treated Pf3D7 and Pf140/SS (P<0.01).

Conclusions: Noscapine has antimalarial properties comparable to standard antimalarial DHA with better safety profiles, which may be considered a therapeutic candidate for the treatment of malaria.

KEYWORDS: Malaria; *Plasmodium falciparum*; Noscapine; Antimalarial; Dihydroartemisinin; Cytotoxicity; Falcipain-2

1. Introduction

Plasmodium falciparum (*P. falciparum*) infection and the associated malaria mortality is a major public health concern mostly in the tropics. According to World Malaria Report in 2022, malaria remained a substantial worldwide health obstacle, with almost 249 million cases documented in 85 countries and territories. This represented an unpleasant increase of 5 million instances in comparison to the preceding year[1]. The estimated number of malaria-related deaths as per World Health Organization in 2021 was 619000[2]. Startlingly, the malaria mortality rates have been steady, despite the patients receiving the most effective antimalarial(s)

Significance

Given the emergence of artemisinin resistance across the tropical world, the timely introduction of newer and safer antimalarials is paramount. Native noscapine is known for its widely recognized therapeutic applications; however, there is a lack of reports on the evaluation of its antimalarial efficacy compared with the standard drug and its mechanism of action. Noscapine exhibited comparable antimalarial properties and better safety profiles than dihydroartemisinin in *in vivo* and *in vitro* studies, suggesting its antimalarial therapeutic potential.

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like artesunate, and with optimal clinical management provided in even resource-rich settings. Antimalarial drug resistance has recently become a major challenge in the fight against malaria worldwide. Resistance towards artemisinin and its derivatives which are the front-line antimalarial drug owing to the mutations is concerning[3–5]. There have been previous reports of late artemisinin based combination therapy (ACT) failure recorded in Southeast Asia including Northeastern India[6,7]. *P. falciparum* has also been found resistant to quinolones, and the antifolate family of antimalarial drugs, which is another major obstacle to achieving the success of numerous in-country malaria elimination programs[7].

The first antimalarial medicine identified from *Cinchona* bark is alkaloid quinine[8]. Artemisinin, the first-line life-saving drug, is a natural endoperoxide that was derived from the sweet wormwood plant *Artemisia annua* L.[4]. Subsequently, numerous antimalarial compounds with promising activities *e.g.*, cyclopeptide, quinoline, pyridocoumarin, acridone, and macrocyclic alkaloids were identified. However, the evolution of drug resistance and its gradual phylogenetic migration all over the globe, has hampered its utility in the current malaria treatment. Thus, there is an urgent need for the discovery of newer and safer antimalarial compounds. Noscapine is a naturally isolated alkaloid and a member of the benzylisoquinoline alkaloid group that has enormous therapeutic activity as per several studies[9]. Its antitussive activity was discovered in 1930, and this alkaloid has been widely utilized as a cough suppressant since the 1960s due to its low toxicity and non-narcotic nature[10]. The oral bioavailability of noscapine has been well defined and it is considered as a potentially effective phytocompound with several medicinal uses in humans. Noscapine has demonstrated potent anti-cancer effects, as these alkaloids limit cell growth by targeting the microtubules[11]. Remarkably, several studies have shown that noscapine has anti-inflammatory properties and can lower the levels of proinflammatory cytokines like IL-1β, IFN□γ, and IL-6[12]. Furthermore, it has been observed that noscapine and its derivatives have antibacterial effects against a number of clinically significant pathogens[13]. Recent research has also revealed that noscapine and its analogs, such as 9-bromonoscapine, amino noscapine, and 9-nitronoscapine, can be utilized to treat polycystic ovarian syndrome, stroke, and other conditions[14].

The *P. falciparum* falcipain-2 (PfFP-2) is a type of enzyme classified as a cysteine protease that breaks down hemoglobin inside the parasite-infected red blood cells (RBCs,) specifically during the trophozoite development stage. Blocking the PfFP-2 hinders the development of parasites, making it an ideal target for antimalarial medications[15]. Previous studies suggest that a variety of inhibitors including peptides, peptidomimetics, and chemotypes such vinyl sulfones, halomethyl ketones, aldehydes, isoquinolines, thiosemicarbazones, and chalcones, have been developed to specifically target PfFP-2[16]. In a recent study, the possible mechanism of action of noscapine against PfFP-2 protein was predicted using molecular dynamic simulation studies[17]. Nonetheless, the experimental evaluation of the mechanism has never been validated as of date. In the present study, we aimed to evaluate the antimalarial activities of noscapine against the *P. falciparum* 3D7 strain (Pf3D7), clinical isolate (Pf140/SS), and *Plasmodium berghei* ANKA (PbA), in comparison with a standard antimalarial drug dihydroartemisinin (DHA) using *in vitro* and *in vivo* models, as well as to explore the mechanism of action of noscapine against falcipain 2.

2. Materials and methods

2.1. Ethical approval

Permission to carry out this work and ethical clearance was obtained from the Institutional Ethics Committee (IEC), CWS hospital, Rourkela under the approval Ref no. (IEC CWSH/013/2022). All experiments involving laboratory animal studies were performed as per the Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines and the Institutional Animal Ethical Protocols. The animal experimentation methods were evaluated and accepted by the Animal Ethical Committee of Sambalpur University under approval Ref no.: (SU/BTBI/IAEC/2023/04).

2.2. Sample collection

Venous blood sample from a severe malaria patient [diagnosed with *P. falciparum* histidine-rich protein 2 (Pfhrp2) RDT+ and microscopically confirmed (having 9% peripheral parasitemia) with severe jaundice (serum bilirubin: 3.9 mg/dL)] was collected at the Department of Molecular Biology and Infectious Diseases, Community Welfare Society Hospital, Rourkela, Odisha, India after obtaining a written informed consent form. Infected blood aliquots were stored in a malaria bio-repository (−80 ℃).

2.3. Characterization and identification of clinically isolated malaria parasite 140/SS, in vitro parasite culture and maintenance

To characterize and identify the species of malaria parasite *P. falciparum* 140/SS, a rapid diagnostic test and light microscopy-based species identification were performed, followed by a modified nested PCR amplification which was performed as described previously based on 18 small subunit rRNA genes[18]. *P. falciparum* 3D7 (Pf3D7) strain was obtained through BEI Resource, NIAID, NIH, USA, and the *P. falciparum* clinical isolate (Pf140/SS) was isolated from a severe malaria patient, for the *in vitro* cultivation by using the Trager and Jensen *in vitro* culture techniques (1976)[19]. Parasites were cultivated in type O^+ erythrocytes and culture medium RPMI-1640 enriched with 10% O^+ human serum, 25 mM HEPES, 10 mM

hypoxanthine, and 50 μg/mL gentamycin and maintained at 37℃ under an atmosphere of 5% carbon dioxide, 5% oxygen, and 90% nitrogen. Parasite growth was monitored every 24 h during the daily replacement of the culture medium. Parasitized RBCs (pRBCs) were stained with 10% Giemsa staining solution and subsequently after that parasitemia was calculated as a percentage based on the viable parasitic forms observed by counting at least 2000 erythrocytes.

2.4. In vitro ring–stage survival assay (RSA)

Pf3D7 and the culture-adapted clinical isolate (Pf140/SS) were synchronized with 5% sorbitol (Sigma-Aldrich). The parasite schizonts containing 10 to 12 nuclei were incubated for 15 min at 37℃ in RPMI-1640 (Gibco) supplemented with sodium heparin, then purified on a 75% Percoll (Sigma-Aldrich) by density gradient centrifugation, washed with RPMI-1640, and cultured for 3 h with freshly supplemented erythrocytes to eliminate any lingering schizonts. RSA was immediately carried out after cultures were treated with 5% sorbitol with 0-3 h post-invasion rings. In this experiment, parasites were plated in 24 well culture plates and treated with 200 μg/mL (483.77 nM) of commercially available noscapine (Sigma Aldrich) as test substance, 200 μg/mL (708.39 nM) of DHA (Sigma Aldrich) as the positive drug control, or 0.1% dimethyl sulfoxide (DMSO, Sigma Aldrich) as the control for 6 h, then washed with RPMI-1640 to remove drug and refilled with RPMI-1640 complete media containing 10% heat-inactivated O^+ serum, 50 μg/mL gentamicin, and grown at 37℃ in a CO_2 incubator. Blood smears were prepared and stained with 10% Giemsa for 20 min. Survival rates were calculated from the percentage of living parasites that formed second-generation rings or trophozoites with normal morphology at 66 h (RSA 0-3 h) after drug withdrawal. About 10000 erythrocytes were examined in each sample by two microscopists (SC and SM) who were not aware of what the other person found. The mean value of live parasitemia was used to show the survival rates of parasites exposed to noscapine, DHA, and 0.1% DMSO[20].

2.5. In vitro antimalarial activity by SYBR green assay

The *in vitro* antimalarial assay was evaluated by SYBR green fluorescent assay. In brief, test compounds were subsequently diluted with RPMI-1640 complete culture medium at different concentrations of two-fold serial dilutions into a 96-well microtiter plate ranging from 200 nM to 0.78 nM to treat in a dose-depended manner. DHA was used as a standard reference compound in the same concentration for the comparison study. The volume of each drug in the wells was 100 μL. A volume of 100 μL of inoculum (parasitized erythrocytes 0.5% to 1% and 2% hematocrit suspension) was added to each well to reach a final volume of 200 μL. Therefore, wells with parasitized erythrocytes and without test compounds served as negative controls whereas wells containing cultures with DHA served as positive controls. The antiplasmodial assay was carried out in duplicate. Plates were confined in a CO_2 incubator for 72 h. After 72 h of incubation, the plates were removed from the incubator and frozen at −20℃. After thawing of the 96-well plates, 100 μL of the cell suspension of each well was dispensed in a new 96-well plate containing 100 μL of SYBR Green I fluorescent lysis buffer was prepared by dissolving 5 μL of SYBR Green I (Himedia) with lysis buffer prepared by Tris-Base, 0.93 g of EDTA, 40 mg of saponin, and 400 μL of Triton X[21]. After that, the plate was incubated for 1 h at room temperature in the dark. The SYBR Green fluorescence was measured with a fluorescence microplate reader (FLUOstar Omega) at the excitation and emission wavelengths of 485 nm and 528 nm, respectively[22]. IC_{50} values were estimated from curves plotted of relative fluorescence units with different concentrations of noscapine and DHA (GraphPad Prism, version 10.2.1).

2.6. IC_{80} phenotypic assay

Parasite cultures were synchronized using 5% sorbitol and the cultures were then diluted to reach a final volume of 200 μL with 6% parasitemia and 2% hematocrit with all early and mature stages in a complete medium. After 72 h of treatment with noscapine and DHA at their respective IC_{80} concentrations, images were analyzed[23].

2.7. In vitro hemolysis assay

The cytotoxic effect of noscapine on RBCs was investigated using a modified hemolysis assay, as described before[24]. Fresh human RBCs were obtained from a healthy volunteer. Then the blood was washed three times with sterile phosphate-buffered saline (PBS) (pH 7.4). A hemolysis assay was conducted by taking 2% hematocrit with various concentrations of the drug and the compound ranging from (12.5 to 1200 nM/mL) in a final volume of 0.3 mL, followed by 24 h of incubation at 37℃. As a positive control, Triton X-100 (1% in PBS) was employed, whereas 0.2% DMSO was used as the negative control[24] After incubation, the tubes were centrifuged at 1000 ×*g* for 10 min, and the absorbance of the supernatants was measured at 540 nm in a microplate plate reader (Thermo Scientific) to measure the amount of hemoglobin released upon RBC lysis. The experiment was performed in triplicates. Percentage hemolysis was calculated by the following formula.

% Hemolysis =[(Absorbance of the test sample) − (Absorbance of diluent)]/[(Absorbance of positive control) − (Absorbance of diluent)]×100

2.8. In vitro cytotoxicity of noscapine

Murine macrophages (J774.A.1 cells) that were obtained from the NCCS Pune were cultured and maintained in DMEM medium (Sigma, Aldrich) containing 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin (Sigma Aldrich). The cells were incubated at 37℃ in a humidified atmosphere containing 5% CO_2. The cytotoxicity was determined by the Alamar Blue method[25].

Briefly, adherent cells (5×10^3 cells/well) were grown in 96-well tissue culture plates and exposed to noscapine (25 to 800 nM/mL) for 72 h. After incubation, 10% Alamar blue (Invitrogen) was added to the culture plate by replacing fresh media and further incubated in the dark for 4 h at 37℃. Absorbance was measured at 570 nm and 600 nm for reference wavelength and expressed as a percentage of the cells in the control after background absorbance was subtracted. The assay was performed in triplicates. The IC_{50} values were obtained by a non-linear regression equation analysis. The selectivity index (SI) of the test samples was calculated using the formula: $SI = CC_{50}/IC_{50}$.

2.9. In vivo antimalarial activity of noscapine

2.9.1. Experimental animals

The Wister albino rats of both sexes weighing 120 to 150 g were obtained from NIN Hyderabad, India. The rats were housed at room temperature of (25±1)℃, with a 12-hour light/dark cycle. Their primary source of food was commercial food pellets, and they had unrestricted access to clean water at all times.

2.9.2. Administration of malaria parasites

PbA was obtained from the Malaria Research and Reference Reagent Resource Center (MR4), through BEI Resource, NIAID, and NIH. The frozen suspension was thawed in a water bath maintained at 37℃ and then injected 300 μL intraperitoneally (*i.p.*) into Wister albino rats. The degree of parasitemia was tracked daily by microscopically examining Giemsa-stained blood films. After the parasitemia increased in proportion to 10%-20%, the serial passage was performed. For each animal, 1×10^7 parasitized erythrocytes diluted with saline water were chosen for intraperitoneal injection after a heart puncture and blood draw.

2.9.3. Determination of parasitemia

The blood from the tails of rats infected with PbA was collected onto microscope slides. Each slide was then fixed with methanol, air-dried, and then stained with 10% Giemsa solution at room temperature for 20 min. Parasitemia was calculated by counting parasitic erythrocytes under a light microscope equipped with a 100× oil immersion lens, and the results were then used to form a percentage as per the following formula.

% Parasitemia = Number of parasitized erythrocytes/Total number of erythrocytes×100

2.9.4. Evaluation of in vivo antimalarial activity

The rats were randomly divided into four groups of six animals in each group (n=6) for the 4-day suppressive experiments as per the methods[26] with slight modifications[27,28]. Group 1 (uninfected control group) received only distilled water. Group 2 (the negative control group) received solvent (a mixture of 10% Tween 80, PBS, distilled water, and DMSO less than 1%). Group 3 (the positive control group) received 20 mg/kg of DHA, and Group 4 was given noscapine at a dosage of 20 mg/kg of body weight on the 5th, 6th, 7th, and 8th day of infection. Drugs were administered using oral gavage. The parasitemia was monitored every morning before food taking and the percentage of inhibition in parasitemia was calculated by using the following formula after treatment.

% Inhibition = [Parasitemia (negative control) − Parasitemia (treated group)]/Parasitemia (negative control)×100

2.10. Determination of PfFP–2 expression using PCR assay in vitro

The effect of noscapine on the cysteine protease of *P. falciparum* was measured by determining the mRNA expression of *PfFP–2* in parasites using quantitative real-time PCR, as per earlier methods[29]. Briefly, from the treated and untreated sample, total RNA was isolated using TRIzol reagent (Invitrogen) and was purified with an RNeasy mini kit (Qiagen, Germany). cDNA was synthesized using the superscript Ⅲ cDNA synthesis kit (Invitrogen, USA) as per instructions. Quantitative real-time PCR was performed using $2^{-\Delta\Delta Ct}$ method with SYBR Green PCR Master Mix in a QuantStudio-5 Real-Time PCR (Applied Biosystems). The primers used in the PCR analysis were *FP–2* forward 5'GCTTGTAGGTTTTGGTATGAAAGAA-3' and reverse 5'-AGATAGGTCCCTTTTTAAAATACTATTGAC-3' with the housekeeping gene 18S rRNA (forward primer 5'-TTTGATGCTTATATTTTGCATACTTTTC-3' and reverse primer 5'-ACAATTCATCATATCTTTCAATCGGTA-3'). The amplification protocol consisted of initial denaturation for 10 min at 95℃, followed by 45 cycles of denaturation at 95℃ for 15 s, and annealing at 60℃ for 1 min.

2.11. Statistical analysis

All assays were carried out in triplicates. The data were expressed as mean ± standard deviation (SD), and statistical significance was determined at a P-value of < 0.05, using one-way analysis of variance (ANOVA). Data from dose-response experiments were represented as the percentage of inhibition compared with the control and analyzed with Prism™ (GraphPad Prism 10.2.3).

3. Results

3.1. Survival rate of parasites after treatment with noscapine

The findings of the light microscopy, rapid diagnostic test, and nested PCR experiment confirmed the species of the clinical isolate to be *P. falciparum* Pf140/SS (sensitive to DHA). The antimalarial activity of noscapine was evaluated against the lab-adapted strains Pf3D7 and clinical isolate Pf140/SS. It was shown that the percentage survival of the parasites exposed to noscapine was (5.80±1.18)% in Pf3D7 compared to the untreated control, which was similar to DHA (4.97±0.34)%. As for the clinical isolate (Pf140/SS), the survival percentage was (7.89±0.43)% after treatment with noscapine and (8.01±0.30)% with DHA (Figure 1).

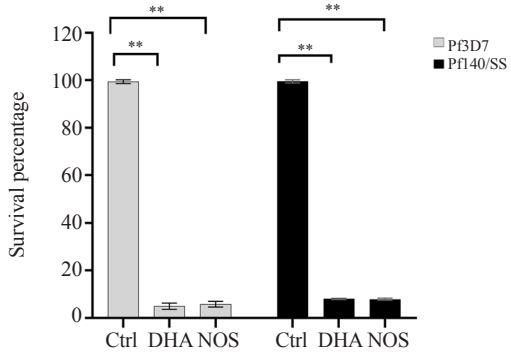

Figure 1. Ring stage survival percentage after treatment with noscapine. $^{**}P<0.01$ compared with the negative control. Ctrl: negative control; NOS: noscapine; DHA: dihydroartemisinin.

3.2. SYBR green assay results

The IC_{50} values of noscapine and DHA obtained were (7.68±0.88) nM/mL and (4.62±0.66) nM/mL, respectively for Pf3D7 and for Pf140/SS, they were (5.57±0.74) nM/mL and (4.06±0.60) nM/mL, respectively (Table 1).

3.3. Multi–stage specific action of noscapine

IC80 phenotypic assay showed that noscapine could inhibit the growth of the ring, trophozoite, and schizont stages of *P. falciparum*, a phenotype that was also found with the standard drug DHA. When noscapine was administered to the *P. falciparum* ring stage parasite, we noticed larger vacuoles close to hemozoin and many aberrant vacuoles in mature parasites, as well as the inability of the parasites to emerge both in Pf3D7 (Figure 2A) and clinical isolate Pf140/SS (Figure 2B).

3.4. Effect of noscapine on hemolysis

Erythrocytes from human volunteers were used to compare the hemolytic activity of noscapine to that of DHA using established techniques. The subsequent release of hemoglobin was utilized to evaluate hemolytic activity as a function of noscapine and DHA concentration, with concentrations ranging from 12.5 nM/mL to 1 200 nM/mL. These dose-response results are shown in Figure 3A. Noscapine demonstrated significantly less hemolysis in human blood than DHA. After 24 h of treatment, it only had (19.71±0.45)% hemolysis, while DHA had (49.65±0.23)%.

3.5. Effect of noscapine on cytotoxicity

After 72 h of drug exposure, noscapine did not cause any damage to the cells, even when they were exposed to very high concentrations of the drug (Figure 3B). In addition, the CC_{50} values obtained by non-linear regression was 1 156 nM/mL, indicating no toxicity was seen (Table 1). In J774.A.1 cells, the selectivity index of noscapine was 150.52 for the Pf3D7 and 207.54 for the clinical isolate (Pf140/SS). A higher selectivity index indicates a stronger inclination towards targeting malaria parasites rather than host cells. This is

Table 1. *In vitro* antimalarial activity of noscapine on Pf3D7 and its clinical isolate Pf140/SS by SYBR green assay.

Agents	IC_{50} (nM/mL)		CC_{50} (nM/mL)	SI value	
	Pf3D7	Pf140/SS		Pf3D7	Pf140/SS
DHA	4.62±0.66	4.06±0.60	545.30±3.06	118.03	134.34
Noscapine	7.68±0.88	5.57±0.74	1 156.00±2.73	150.52	207.54

IC_{50}: concentration of the agent at which 50 % inhibition of the parasites is achieved. CC_{50}: concentration at which 50% of cells are killed. SI: selectivity index (CC_{50}/IC_{50}). DHA: dihydroartemisinin.

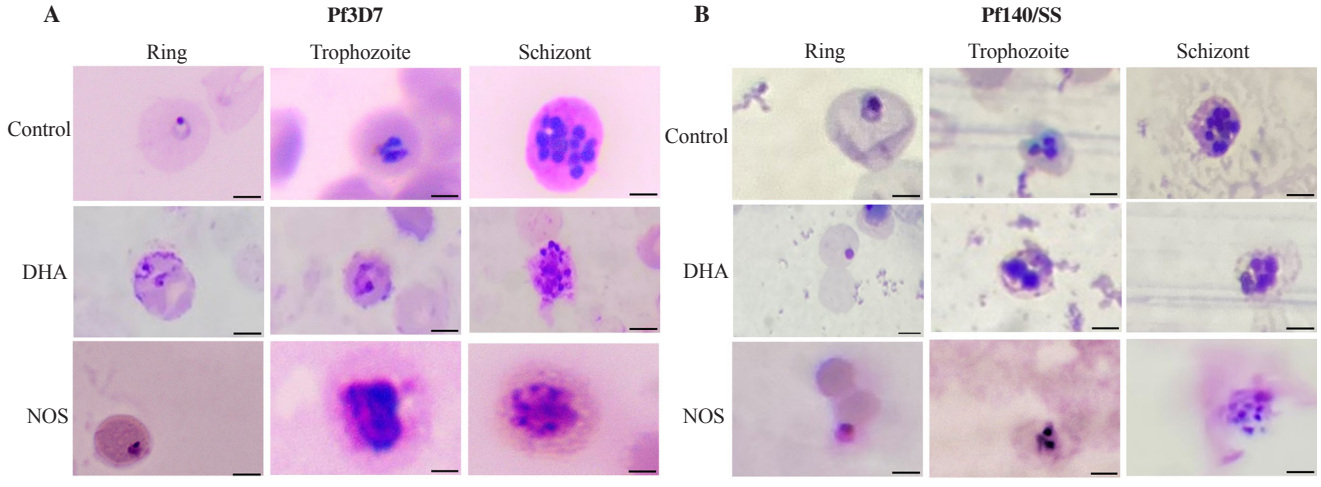

Figure 2. Microscopic visualization of different *Plasmodium falciparum* stains treated with IC_{80} concentrations of tested compounds. (A) Pf3D7 and (B) clinical isolate strain (Pf140/SS) with and without drug treatment at the ring and mature stages (magnification: 1 000×; scale bar = 5 μm).

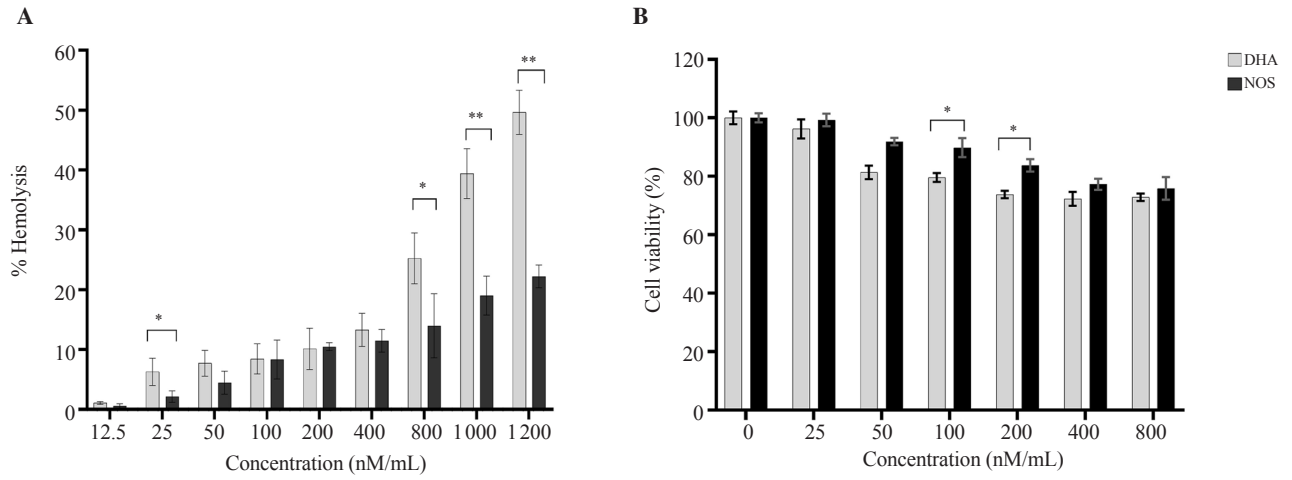

Figure 3. *In vitro* assessment of (A) hemolysis profile and (B) cytotoxic effects of noscapine. Data are presented as mean ± SD of triplicate experiments. $^{*}P<0.05$, $^{**}P<0.01$.

advantageous since it decreases toxicity and enhances the efficacy of the treatment.

3.6. In vivo antimalarial activity of noscapine against PbA

To investigate the progression of PbA in rats, parasitemia monitoring was performed. Parasites in the blood were originally found on day one following infection and the percentage of parasitemia was nearly one percent. On day 8 following infection, it reached 36.81% whereas treatment with noscapine gradually decreased parasitemia in the infected rats with an inhibition rate of 95.19%, similar to that of DHA (Figure 4).

3.7. Effect of noscapine on the mRNA expression of PfFP–2

Treatment with noscapine significantly downregulated *PfFP–2* mRNA expression compared to untreated controls in both Pf3D7 ($P<0.01$) (Figure 5A) and Pf140/SS ($P<0.01$) (Figure 5B). Its effect was less significant than that of DHA ($P<0.05$).

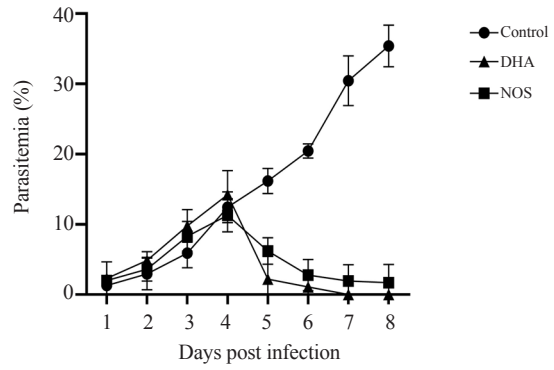

Figure 4. Antimalarial activity of noscapine in *Plasmodium berghei* ANKA-infected rats. Data are presented as mean ± SD of three experiments.

4. Discussion

Noscapine, a natural alkaloid molecule found in opium poppy (*Papaver somniferum*), has been considered a promising drug with diverse clinical uses[30]. Based on the recommendations from Royal Commission on Opium, noscapine was widely used as an antimalarial in the Indian subcontinent both for prophylaxis and treatment, from the years 1895 to 1930 though subjected to reasonable criticism[31]. Nonetheless, native noscapine isolated from opium remained a subject of considerable interest in medical and pharmacological studies due to its wide-ranging therapeutic potential. The present study highlights its antimalarial properties using *in vitro* and *in vivo* models, as well as safety using cytotoxicity and hemolysis profile *in vitro*.

Noscapine was tested for antimalarial activity *in vitro* against Pf3D7, and compared with that of Pf140/SS, a clinically isolated severe malaria strain. *In vitro* antimalarial evaluation against Pf3D7 and Pf140/SS revealed that noscapine has potential antimalarial activity, with IC_{50} of (7.68±0.88) and (5.57±0.74) nM/mL, respectively for both strains which is nearly similar to DHA [IC_{50}=(4.62±0.66) nM/mL and (4.06±0.60) nM/mL]. Recent studies have explored the antimalarial potential of various alkaloids, showcasing their diverse applications in the fight against malaria. Notable examples include the historically significant quinine derived from the cinchona tree, and artemisinin, a key component in modern artemisinin-based combination therapies[32]. Cryptolepine, derived from the *Cryptolepis sanguinolenta* shrub, has exhibited promising antimalarial activity[33]. Alkaloids from the Madagascar periwinkle, such as vincristine and vinblastine, known for their anticancer properties, have also demonstrated efficacy against malaria[34]. Additionally, plant-derived alkaloids like ajmalicine and serpentine[35] berberine[36], and coptisine[37] have been investigated for their potential antimalarial effects.

The present study also evaluated the *in vivo* antimalarial effect

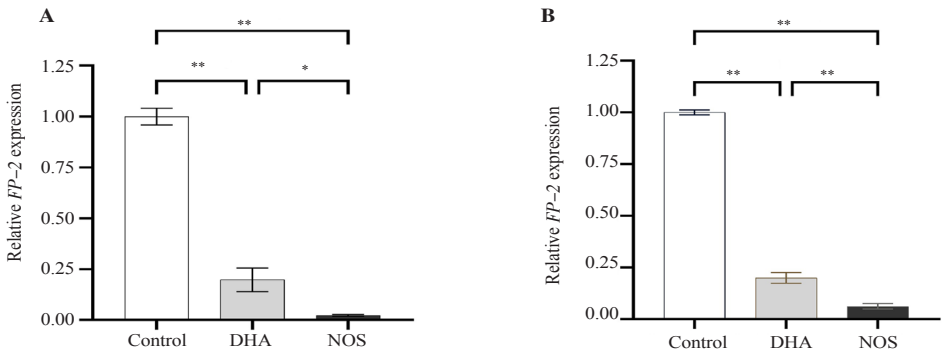

Figure 5. RT-PCR analysis of *PfFP–2* mRNA levels after treatment with noscapine. Data are presented as mean ± SD of triplicate experiments. $^{*}P<0.05$, $^{**}P<0.01$. PfFP-2: *Plasmodium falciparum* falcipain-2.

of this natural alkaloid compound noscapine in PbA-infected rats. Noscapine treatment showed a 95% reduction in parasitemia. Earlier studies have reported that compounds showing a 30% reduction of parasitemia *in vivo* are considered to have active effects[38]. Thus, this compound may be considered an active antimalarial compound based on the current findings from *in vitro* and *in vivo* experiments.

Previous studies have also shown that noscapine has no cytotoxic effect on normal human embryonic kidney cells (HEK293) which is <5%, even at 100 μM[39]. This study examined the cytotoxic effects on RBCs and in murine macrophage cell lines (J774.A.1), showing no or very little cytotoxicity even at very high concentrations, which is better than the standard antimalarial DHA. We also show that noscapine results in very little hemolysis unlike the hemolysis profiles of the standard DHA (more than two-fold) which is already known earlier to cause severe and delayed hemolysis (mild to moderate anemia) accompanied by fever and body swelling[40,41].

Based on the study findings and the differences in the morphological changes in parasites before and after noscapine treatment, the gene expression study demonstrated the mechanism of action of noscapine in the parasitized RBCs. Molecular dynamics, structural alignment, and free energy studies have also indicated that noscapine may have a comparable biological function targeting falcipain 2[17]. The falcipain 2 gene was highly downregulated as compared to the control in Pf3D7 and Pf140/SS. Various studies have shown that the drug candidate targeting falcipain 2 is the most prominent drug[42]. In this study, it was confirmed noscapine can inhibit *PfFP–2* mRNA expression, and and thereby suggesting its possible role in blocking hemoglobin hydrolysis inside infected RBCs and suppressing overal parasite growth[17]. Falcipain-2 is the papain-like cysteine protease of *P. falciparum* localized in the food vacuole, and responsible for cleaving the membrane skeletal proteins during late parasite development. In the current malaria pre-elimination era, existing antimalarial drugs have varying levels of efficacy against different species and stages of malaria parasites and present several limitations. Malaria parasites of humans over time, have demonstrated a remarkable ability to develop resistance to existing antimalarial drugs. This resistance gradually reduces the efficacy of current treatment options like DHA and combinatorial drugs, thereby posing a significant challenge to malaria control and elimination efforts. Timely introduction of novel antimalarial compounds like native oscapine, with novel mechanisms of action as hypothesized above, may help overcome the issues concerning drug resistance and provide effective therapeutic alternatives[43–46]. In addition, novel plant-derived bioactive molecules like morphinans (*e.g*. tazospine) and alkaloids (*e.g*. noscapine), may provide new avenues to target different stages of the malaria parasite's life cycle, including the liver stages[47]. However, there are some apparent limitations of this study, *e.g*. the reliance of the study findings on *in vitro* assays and animal (mouse) models to assess the effectiveness of noscapine as a potent antimalarial agent. Although these standard models have been in extensive use in malaria research methods worldwide and offer significant insights, they may not fully reproduce or mimic the intricate nature of the malaria infection scenario and are often considered preliminary findings. Accordingly, additional *in vivo* assessments and clinical trials will be necessary to demonstrate the efficacy and safety profile of noscapine as an antimalarial and evaluate possible adverse effects.

In conclusion, noscapine shows *in vitro* and *in vivo* promising antimalarial properties with a safe profile The targeted inhibition of PfFP-2 by noscapine also indicates its therapeutic potential. Future evaluation is needed to verify the efficacy of noscapine in severe malaria.

Conflict of interest statement

The authors declare no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

PKS and PKN conceptualized the study. SKB performed the experiments and investigation with inputs and support from SM, SC, and DRS. AN, SK, SM, PKB, PKN, and PKS monitored and supervised the experiments on-site. PKN, PKS, AN, and SK provided the resources. SKB and PKS did the formal analysis. PKS and PKN supervised the study. SKB wrote the manuscript. PKS edited the manuscript.

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