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Contact exposure to ivermectin induces acute mortality and inhibits parasite development in malaria vectors

Meenakshi Jeena[1,2], Bharti Goyal[1,2], Suman Lata[1,2], Gaurav Kumar[1], Lipun Kumar Pradhan[1], Yamini Thakur[1,2], Gaganpreet Kaur[3], Vaishali Verma[1,2], Kailash C. Pandey[1,2], Shweta Pasi[4]✉ & Jaspreet Kaur[1,5]✉

Resistance to insecticides and associated behavioural shifts are being increasingly reported in malaria vectors. To counter these adaptations, there is a pressing need to explore novel control tools and interventions. In line with this, the present study evaluates the potential of ivermectin as a contact-toxin for both malaria vectors and parasite. Laboratory reared female *An. culicifacies* and *An. stephensi* mosquitoes were exposed to different concentrations of ivermectin through topical and bottle bioassays. Mortality data was used to calculate the LD_{50} and LD_{90} values. Infection studies with *Plasmodium berghei* were done in female *An. stephensi* to check the transmission blocking activity of ivermectin. Following contact exposure to ivermectin, the midguts of exposed mosquitoes were dissected and oocysts were counted to calculate oocyst intensity and prevalence. Ivermectin demonstrated high contact toxicity against both *An. stephensi* and *An. culicifacies* mosquitoes inducing 100% mortality in both vector species within 24–48 h of exposure at higher dosages of ivermectin. In topical bioassay, after 48 h the LD_{50} value for *An. stephensi* and *An. culicifacies* was 0.017 ng/mg (95% CI 0.008–0.30) and 0.002 ng/mg (95% CI 0.000–0.005) respectively. The corresponding LD_{90} values were 0.264 ng/mg (95% CI 0.138–.703) and 0.174 ng/mg (95% CI 0.063–1.173) for *An. stephensi* and *An. culicifacies*. Whereas in bottle bioassay after 48 h, the LD_{50} value for *An. stephensi* and *An. culicifacies* was 4.245 µg/bottle (95% CI 3.018-5.715) and 1.768 µg/ bottle (95% CI 1.211-2.528) respectively. The LD_{90} value of *An. stephensi* and *An. culicifacies* was 13.10 µg/bottle (95% CI 10.56-17.50) and 5.218 µg/ bottle (95% CI 4.02-7.63). Additionally, contact exposure to ivermectin significantly impaired oocyst development in mosquitoes. A reduction of 71% in oocyst numbers was observed at 0.01 µM concentration of ivermectin. Our study establishes ivermectin as an effective contact mosquitocidal and transmission blocking agent. These findings further contribute to the growing body of evidence supporting the use of ivermectin as a novel vector control tool capable of simultaneously reducing vector population and interrupting malaria transmission.

Keywords Ivermectin, Contact mortality, Malaria transmission, Plasmodium oocyst, Infection, Bottle bioassay

Malaria remains a significant global health challenge with an estimated 263 million cases and 5,97,000 malaria deaths worldwide in 2023[1]. Vector control is the cornerstone of malaria management which relies heavily on the use of insecticide-based interventions like insecticide residual sprays and insecticidal nets in affected areas. These strategies leverage the indoor resting and feeding behaviour of mosquitoes and include insecticides of chemical classes carbamates, neonicotinoids, pyrethroid organochlorines and organophosphates as their active ingredients. However, the extensive and prolonged use of insecticides in the last few decades, has led to the

[1]ICMR-National Institute of Malaria Research, Sector-8, Dwarka, New Delhi 110077, India. [2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India 201002. [3]Post Graduate Department of Chemistry, Multani Mal Modi College, Punjabi University, Patiala, Punjab, India. [4]Molecular Biology Division, ICMR-National Institute of Nutrition, Beside Tarnaka Metro Station, PO Jamia Osmania, Hyderabad, Telangana 500007, India. [5]Faculty of Biological Sciences, Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India. ✉email: shweta.pasi@gmail.com; shweta.pasi.nimr@gov.in; kaur.jaspreet0708@gmail.com; drjaspreet.kaur@icmr.gov.in

development of insecticide resistance among mosquito vectors[2]. Compounding this issue are the behavioural shifts like altered feeding times, increased outdoor biting and shifts in resting preferences, that mosquitoes adapt to avoid contact with treated surfaces which further undermine the effectiveness of these interventions[1].

The major malaria vectors in India, *Anopheles stephensi* and *An. culicifacies* have reported resistance to multiple classes of insecticides in the several endemic districts of the country[3]. Notably, *An. baimaii,* a predominant malaria vector in the Northeast India, has shown a concerning trend of increased outdoor biting[4]. Further, shifts in resting behaviour of *An. fluviatilis* and *An. culicifacies* from endophily (indoor resting) to exophily (outdoor resting) have also been emerged recently[5]. Given the challenge of insecticide resistance and emerging behavioural adaptations among mosquito vectors, it is imperative to explore novel molecules, innovative tools and integrative strategies for vector control. These developments are essential for mitigating the dual threat of insecticide resistance and residual transmission.

Ivermectin is a widely used veterinary antiparasitic drug. It has found its way into malaria management during mass drug administration for onchocerciasis and lymphatic filariasis in the African continent. Ivermectin has been demonstrated to significantly reduce mosquito populations in areas of MDA[6,7]. Ivermectin has been shown to reduce mosquito lifespan and impaired reproductive output[8,9]. Not only this, ivermectin also blocks the development of malaria parasite within mosquitoes when they feed on drug treated humans[10]. Ivermectin targets the glutamate-gated chloride ion channel in the muscle and nerve cells resulting into paralysis and death of the treated mosquitoes[11]. Due to its strong safety profile and effectiveness against most malaria vectors, ivermectin is the leading endectocide candidate for malaria control. Beyond its use in MDA, the efficacy of ivermectin has been highlighted as an oral toxin in attractive toxic sugar baits (ATSB) formulations, which is a novel vector control tool[12].

While the systemic effects of ivermectin on mosquitoes through ingestion via treated human blood or ATSB formulations are well established, its impact on mosquito survival through contact exposure remains underexplored. Recently, a study has documented the mosquitocidal effects of ivermectin through treated surfaces against African malaria vector *An. gambiae*[13]. While resting on a treated surface, the cuticle on the legs and appendages of the insect are the initial points of contact that also form the gateway for the entry of xenobiotics. The mosquito cuticle contains a significant amount of lipids in the form of cuticular hydrocarbons[14]. Since ivermectin is a highly lipophilic drug[15], we hypothesized that its high lipophilicity may facilitate its uptake through the tarsi of mosquitoes when exposed to treated surfaces. To test this, we first determined the intrinsic toxicity of ivermectin through topical bioassays. We then coated glass bottles with different concentrations of ivermectin and assessed the effects of contact exposure of ivermectin on the survival of two major Indian vectors: female *An. stephensi* and *An. culicifacies*. We further studied the transmission blocking effects of contact ivermectin on the development of rodent malaria parasite *Plasmodium berghei* in the mosquito midgut.

Methodology

Mosquitoes

Laboratory-reared *Anopheles stephensi* and *An. culicifacies* were maintained at 27 ± 2°C and 70 ± 10% relative humidity (RH) in a dedicated insectary at the ICMR-National Institute of Malaria Research, New Delhi, India. Eggs were hatched overnight in bowls containing deionized water. First instar larvae were counted (150 per pan) and reared in 500 ml of deionized water on a powdered fish food diet (Tetra bit complete fish food diet). Pupae were collected from rearing pans and were transferred to adult emergence cages. Adults were provided with 10% glucose solution and raisins for regular colony maintenance. Mosquitoes were checked for their insecticide susceptibility status using the WHO tube susceptibility bioassay[16].

Compound

Powdered Ivermectin (Sigma Aldrich USA; purity > 94%, CAS Number: 70288-86-7) was dissolved in methanol (Sigma Aldrich) for a stock solution 1 milimolar. The working concentrations were prepared in acetone (BR Biochem, purity > 99%) for assays.

Intrinsic toxicity of ivermectin in *Anopheles* through topical application bioassay

Topical application bioassays were performed to determine the intrinsic toxicity of selected drugs on mosquitoes. The experiments were carried out on three to five days old laboratory reared sugar fed female *An. stephensi* and *An. culicifacies mosquitoes*. Different concentrations of ivermectin solutions ranging from 0.025 to 20 µM were prepared in acetone (Table 1). A constant volume of 0.1 µl of vehicle (acetone) and the drug concentration was applied using a pipette on the pronotum of the thorax of the mosquito[16]. All the tests for the different concentrations were performed in triplicate with 25 mosquitoes per group. After application, all the mosquitoes

Ivermectin concentrations (µM)	Bottle bioassay (µg/bottle)	Topical application assay (ng/mg mosquito)
0.025	0.025	0.0015
0.5	0.437	0.029
1	0.875	0.058
4	3.5	0.233
10	8.75	0.583
20	17.502	1.167

Table 1. Concentrations of Ivermectin used in the bottle bioassay and topical application bioassay.

were transferred to holding cups, provided with 10% glucose-soaked cotton swabs and kept in a climatic chamber at 27 ± 2 °C temperature. After 24 h of holding, the number of dead mosquitoes were counted and the percentage mortality was calculated.

Coating of glass bottles with ivermectin and bottle bioassays for knockdown and mortality

The bioassay was done following the protocol outlined in Common Protocol for Uniform Evaluation of Public Health Pesticides for use in Vector Control, ICMR (Third edition), 2023[16]. Briefly, narrow mouthed glass bottles were coated with different concentrations of ivermectin (Table 1) as test bottles, whereas control bottles were coated with 1 ml of acetone (used as vehicle for diluting ivermectin). After dispensing 1 ml of drug or vehicle solution, the bottles were rolled horizontally on a working table and intermittently rocked vertically to coat the bottle uniformly from the underside of the cap to the bottom of the bottle. The rolling procedure lasted for 15 min or more until the acetone visibly evaporated. These bottles were capped loosely and left overnight for complete drying at room temperature. The number of mosquitoes exposed in each bottle was n = 25. The experiments were carried out on three to five days old laboratory reared sugar fed female *An. stephensi* and *An. culicifacies* mosquitoes. Mosquitoes were introduced into test and control bottles and allowed to rest on the treated glass surface for 60 min and knockdown was recorded. Following exposure, the mosquitoes were transferred into holding cups, provided with 10% glucose solution on cotton swabs and kept at 27 ± 2 °C and 70 ± 10% RH in a climatic chamber. Daily mortality was monitored for each experimental group for the next 48 h.

The bioassay was repeated three times in four replicates for test and two for control, using new batches of mosquitoes and bottles. The test/control mortality was calculated by summing the number of dead mosquitoes across all test/control replicates. This was expressed as a percentage of the total number exposed in test/ control. The test was discarded if mortality in the control group was 20% or above.

Bottle bioassay: The respective tested concentration in microgram was weighed and 1 ml volume was suspended in each test bottle *i.e.*µg/bottle**.**

Topical application assay: Ivermectin, with an average molecular weight of 875.1 g/mol, was tested against mosquitoes. A total of 25 mosquitoes for each batch were weighed and then individual mosquito weight was calculated as total average weight of 25 mosquitoes divided by n = 25.The mean body weight for individual mosquitoes was 1.5 mg.

For dose determination, the quantity of ivermectin in nanograms corresponding to each tested concentration (µM) was calculated using its molecular weight. The drug mass administered per mosquito was determined by multiplying the concentration-dependent drug content per microliter by the applied volume (0.1 µL). To normalize the dose to body size, the calculated drug mass per mosquito (ng) was divided by the mean mosquito body weight (1.5 mg) and expressed as nanograms of ivermectin per milligram of mosquito body mass.

Transmission blocking activity of Ivermectin

Four to six week old BALB/c mice were administered 100 µl phenylhydrazine (10 mg/ml in PBS intraperitoneally on day 0. On day 2, *P. berghei* ANKA between 1 and 10% parasitemia and 10^5 to 10^8 infected erythrocytes was injected intraperitoneally to the mouse[17]. From day 4 of parasite administration, gametocyte count was determined microscopically on a daily basis. Blood droplet from tail vein was smeared onto a glass slide, stained with 1% Giemsa stain and observed under 100 × oil immersions to count the number of gametocytes. When the gametocyte count reached 2–5% (usually by day 8 of infection), infected mice were anesthetized with ketamine (80 mg/kg) and xylazine (5 mg/kg) and mosquitoes were allowed to fed upon them. Three to five days old female *An. stephensi* mosquitoes were starved overnight and then allowed to feed on anesthetized infected mice for 30–45 min (Fig. 1). Thereafter, fully engorged mosquitoes were separated in a cage. Twelve hours after blood feeding, mosquitoes were introduced into drug/vehicle coated bottles (n = 25) and exposed to coated drug/vehicle for 1 h. After contact exposure, mosquitoes were transferred to cages, provided with 10% sucrose solution and maintained at 19 ± 1 °C and 80–90% RH with a photoperiodicity of 12 h L:D in a climatic chamber (Percival Scientific Inc., USA). Around eight days post-infectious blood meal, the midgut of alive mosquitoes in different experimental groups were dissected out and stained with 0.5% mercurochrome. The midguts were observed using light microscopy at a magnification of 10 × and 40 × to determine the presence of oocysts. We calculated oocyst intensity *i.e.* number of oocysts per midgut and oocyst prevalence *i.e.* proportion of mosquitoes infected with oocyst for 0.010 µM drug concentration. However, ivermectin induced significant mosquito mortality, even at low concentration (0.025 µM to 20 µM) with around 25% mortality at lowest dose (0.025 µM). To address this, we reduced the concentration to the nanomolar range (0.010–0.025 µM). Ultimately, we selected 0.010 µM ivermectin for further investigation into its transmission -blocking potential.

The experimental protocol has been approved by Institutional Animal Ethics Committee of ICMR-NIMR with reference No: NIMR/IAEC/2024_1/4, in accordance with international guidelines and regulations. All animal experimental procedures are performed in accordance with ARRIVE guidelines. All surviving infected mice after the successful mosquito feeding were humanely euthanized using the carbon dioxide asphyxiation procedure, in compliance with approved ethical guidelines.

Statistical analysis

The effect of various concentrations of the ivermectin on mosquito mortality was evaluated using descriptive and inferential statistics (Graph Pad prism 8.0.2 version). Mortality data were presented as mean ± standard deviation (SD), with corresponding 95% confidence intervals (CI). A one-way analysis of variance (ANOVA) was performed to assess overall differences among treatment groups, followed by Tukey's test to compare each concentration to the control group. The LD_{50} and LD_{90} value of the topical and bottle bioassay for *An. stephensi* and *An. culicifacies* was calculated using SPSS software (probit analysis). The mean values of the drug replicates (different concentrations) were represented in graphs.

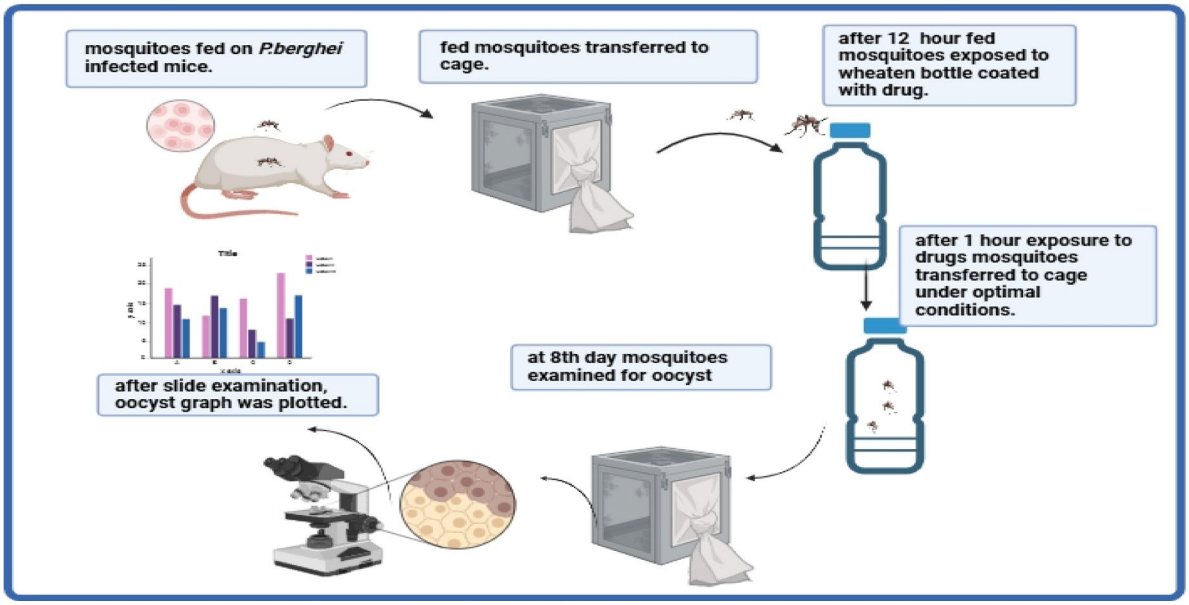


Fig. 1. Schematic of experimental design: Female *An. stephensi* mosquitoes were fed on an infectious bloodmeal from a *P. berghei* infected mice. Following 12 h of bloodmeal, the mosquitoes were exposed to ivermectin coated wheaton bottles for 60 min. After this, the exposed mosquitoes were put into cages and reared at 19 °C and 80–90% relative humidity. Eight days post bloodmeal, the midguts of mosquitoes were dissected out, stained with mercurochrome and examined under the microscope. Oocyst intensity and prevalence were calculated and plotted.

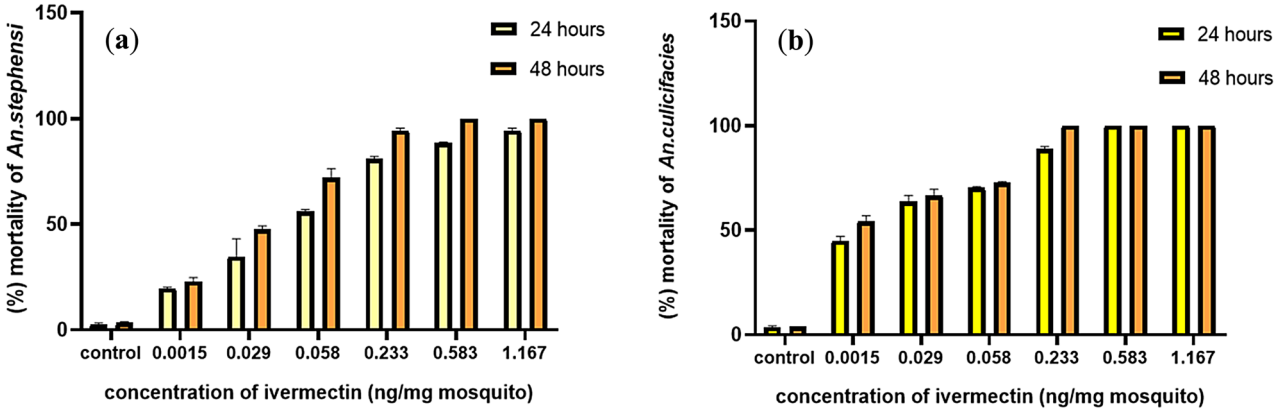


Fig. 2. Ivermectin at different concentrations was applied topically on the pronotum of female (**a**) *An. stephensi* and (**b**) *An. culicifacies*. Acetone (vehicle) was used as control. Mortality was recorded at the end of 24 and 48 h of application and percentage mortality was calculated for each experimental group. Data represents Mean ± SD.

Results

Topical application of ivermectin is highly toxic for *An. stephensi* and *An. culicifacies*

We first assessed the intrinsic toxicity of ivermectin against the major Indian malaria vectors through topical bioassays. Six concentrations in the range of 0.0015 to 1.167 ng/mg mosquito were directly deposited on the thoracic pronotum of *An. stephensi and An. culicifacies* mosquitoes. The control mosquitoes received topical application of vehicle *i.e.* acetone only. Topical application of ivermectin resulted in significantly high mortality at all the tested concentrations in *An. stephensi* and *An. culicifacies,* compared to controls (Tukey's test, $p < 0.0001$). In *An. stephensi*, a dose-dependent increase in mortality was observed with ivermectin (Fig. 2a). The two highest dosages, 0.583 and 1.167 ng/mg-mosquito, were most toxic and induced 100% mortality in treated mosquitoes of both species. The intrinsic toxicity of ivermectin was higher, in *An. culicifacies* upon topical application. The lowest dose tested caused 50% mosquitoes' mortality within 48 h while the higher three dosages, 0.233, 0.583 and 1.167 ng/mg-mosquito induced 100% mortality by 48 h (Fig. 2b). Thus, ivermectin exhibited high intrinsic toxicity against both Indian malaria vectors at the selected dosage range.

Dose–response analysis of ivermectin-topical bioassay after 48 h showed 0.002 ng/mg and 0.174 ng/mg as 50% and 90% lethal dosage (LD_{50} and LD_{90}) values for insecticide susceptible *An. culicifacies*. In the case of insecticide susceptible *An. stephensi*, the LD_{90} value at 48 h was 0.264 ng/mg which was significantly higher in comparison to the LD_{90} for *An. culicifacies*. Similarly, the LD_{50} value after 24 h for *An. stephensi* was 0.028 ng/mg (95% CI 0.013–0.051) and LD_{90} value was 0.671 ng/mg (95% CI 0.317–2.225). Whereas, the LD_{50} value after 24 h for *An. culicifacies* was 0.003 ng/mg (95% CI 0.001–0.009) and LD_{90} value as 0.234 ng/mg (95% CI 0.095–1.101).

Exposure to ivermectin induces acute mortality in *An. stephensi* and *An. culicifacies* upon contact with treated surface

To test whether ivermectin can induce mortality through contact-exposure in *An. stephensi* and *An. culicifacies*, we exposed both species to different concentration of ivermectin coated on the inner surface of glass bottles. The concentrations tested were in the range of 0.025 µM to 20 µM corresponding to 0.025 µg/bottle to 17.502 µg/bottle (Table 1). We observed ivermectin to induce significant mortality at all the tested concentrations in *An. stephensi* compared to controls ($p < 0.0001$). At the lowest dose (*i.e.* 0.025 µg/bottle), the mortality rate remained relatively constant (~25%) with the 24-h and 48-h mortality showing little difference. As the dose increased, a concentration dependent effect was observed on mortality of *An. stephensi* after 48 h of exposure and the highest dose of 17.502 µg/ bottle dosage induced 100% mortality in the experimental group (Fig. 3a). The LD_{50} value after 24 h for *An. stephensi* was 7.234 µg/bottle (95% CI 5.67–9.30) whereas, the LD_{90} value of bottle bioassay was 18.65 µg/bottle (95% CI 15.25–24.40). Similarly, The LD_{50} value after 48 h for *An. stephensi* was 4.245 µg/bottle (95% CI 3.018–5.715) whereas, the LD_{90} value of bottle bioassay was 13.102 µg/bottle (95% CI 10.566–17.505).

An. culicifacies also exhibited dose-dependent mortality from 4.00 ± 0.00% (control) to 100% at ≥ 8.75 µg/bottle. Notable intermediate mortalities included 30.00 ± 2.65% at 0.025 µg/bottle and 77.67 ± 2.52% at 3.5 µg/bottle (Fig. 3b). The LD_{50} and LD_{90} value of bottle bioassay after 24 h for *An. culicifacies* were 3.882 µg/bottle (95% CI 2.347–5.962) and 11.104 µg/bottle (95% CI 8.29–17.315). Similarly, The LD_{50} value after 48 h for *An. culicifacies* was 1.768 µg/bottle (95% CI 1.211–2.528) whereas, the LD_{90} value was 5.218 µg/bottle (95% CI 4.026–7.630).

Tarsal exposure of ivermectin effectively inhibits *P. berghei* oocyst development within *An. stephensi* mosquitoes

We next investigated whether ivermectin can inhibit *Plasmodium* parasite development within mosquitoes via contact exposure. However, ivermectin induced high mortality in mosquitoes in the dose range used in the study (0.025 µM to 20 µM). Even at the lowest tested concentration of 0.025 µM we observed ~ 25% mortality. The high toxicity of ivermectin observed in our study complicated the assessment of the transmission blocking potential of ivermectin. To address this, we reduced the concentration of ivermectin to nanomolar range and evaluated

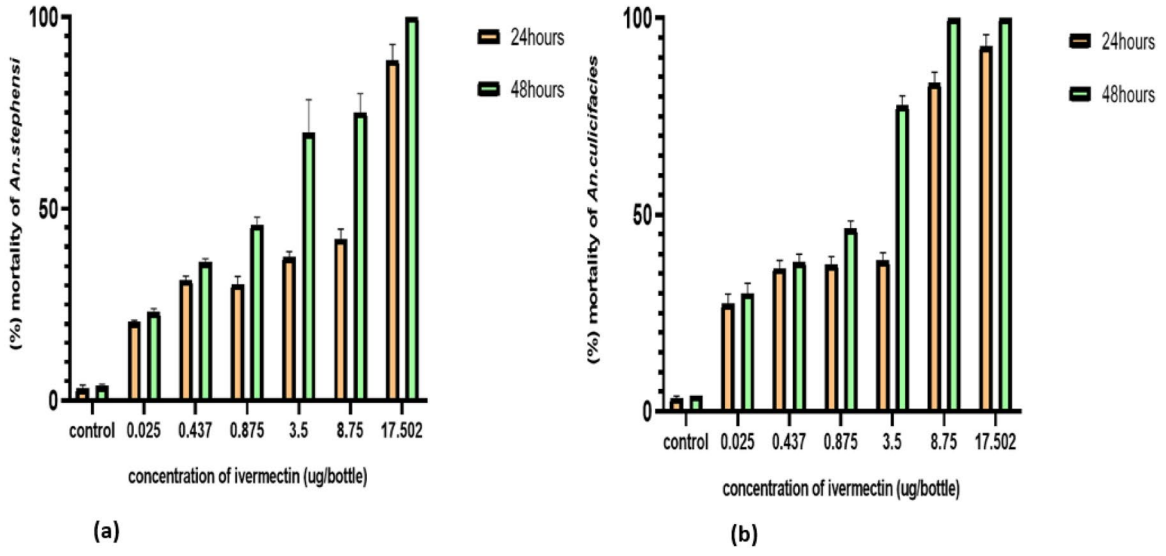


Fig. 3. Female (**a**) *An. stephensi* and (**b**) *An. culicifacies* were exposed to vehicle (acetone) or different concentrations of ivermectin in coated glass bottles for one hour. Mortality was recorded at the end of 24- and 48-h of exposure and percentage mortality was calculated. Data represents Mean ± SD.

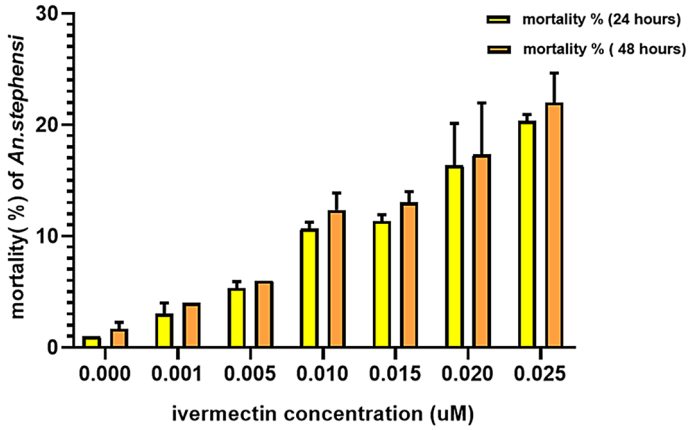
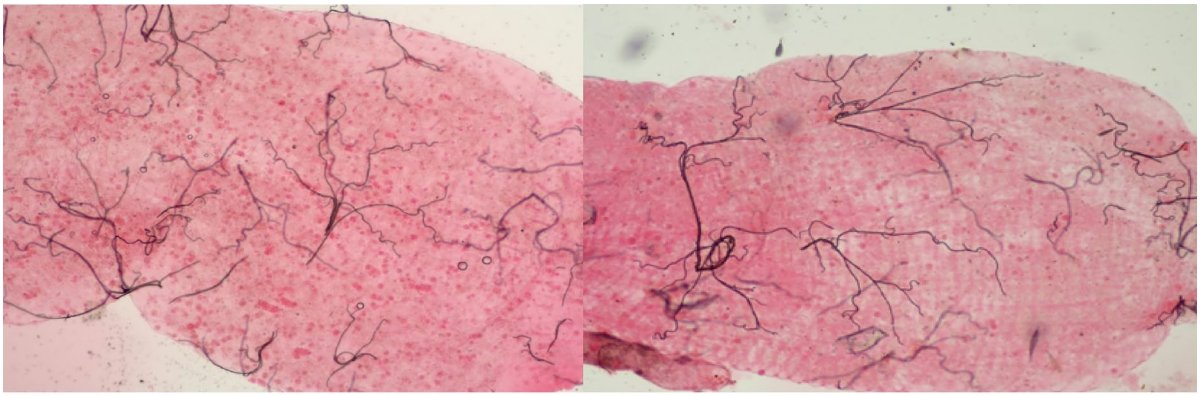


Fig. 4. *An. stephensi* were exposed to nanomolar concentrations of ivermectin for one-hour in coated bottles. Mortality was determined at the end of 24 and 48-h of exposure. Data represents Mean ± SD.



(a) **(b)**

Fig. 5. Micrographs of the midguts of *An. stephensi* infected with *P. berghei* oocysts on day 8 -10 of infectious blood meal. (**a**) Midgut of vehicle exposed mosquito (**b**) Midgut of 0.010 µM ivermectin exposed mosquito. The images were taken at 40X magnification. Oocysts are red spherical structures enclosed by plasma membrane and thick capsule in the midgut of *An. stephensi*.

mosquito mortality. We first determined contact dependent effects of ivermectin on mosquito mortality at 0.001 µM, 0.005 µM, 0.010 µM, 0.015 µM, 0.020 µM 0.025 µM concentrations using bottle bioassays. Even in the nanomolar range ivermectin exhibited a significant contact-dependent toxic effect on exposed mosquitoes. Mortality rates were greater than 15% in the 0.020–0.025 µM range. Mosquito mortality at ivermectin dosages 0.010 and 0.015 µM was significantly less (~ 10%) and thus we selected the 0.010 µM ivermectin dosage to test its transmission blocking potential (Fig. 4). Ivermectin (10 nM) exposed mosquitoes were given infectious blood meal after 12 h of exposure.

Notably, we observed significant reductions of ~ 71% in oocyst numbers (Figs. 5 and 6). The control showed 81% of infection intensity with 72.5% of prevalence. Whereas, at 0.010 µM of ivermectin the intensity of infection was 17.5% with 60% prevalence. The mean number of oocysts/midguts in control group was 73 and in experimental ivermectin group was 21.

Discussion

Novel interventions are crucial not only for vector control but also for inhibiting parasite transmission. The present study highlights the toxic potential of ivermectin in causing mosquito mortality and also disrupting parasite development within them through contact exposure. The other studies also underscore the potential of ivermectin, as a future complementary tool along-side conventional insecticide-based vector control tools, especially in regions with high mosquito density and insecticide resistance. Studies have shown ivermectin as a promising molecule for ATSB[12,18], mass drug administration[6]. Ivermectin decreases in vitro *P. berghei* infection in human hepatic cell line and in vivo, in rodent models[19]. One possible method that our report has elucidated is contact-based effect of the molecule on the mosquitoes' survival and parasite inhibition. To the best of our knowledge, this study has reported for the first time that contact-based exposure to ivermectin causes mortality

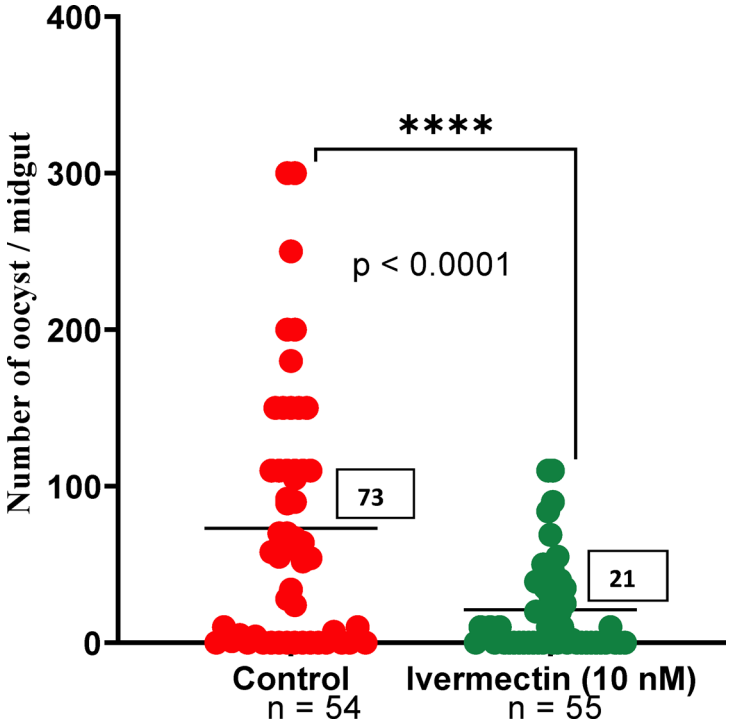


Fig. 6. *An. stephensi* mosquitoes were exposed to 0.010 µM of ivermectin for 60 min in bottle bioassays after an infectious bloodmeal. On day 8 post infectious bloodmeal, the midguts of mosquitoes in control and test groups were dissected and stained with mercurochrome for detection of oocysts. Reduction of infection in terms of intensity and prevalence is mentioned. The transmission blocking activity of the two drugs is represented in terms of oocyst intensity of *P. berghei i.e.* number of oocysts/midguts in different experimental groups. Means are indicated. ****$p < 0.0001$.

among major Indian malaria vectors *An. culicifacies* and *An. stephensi* and the sub-lethal dose of the drug can also inhibit parasite development in mosquitoes through the same route.

Our study is in-line with the earlier studies, whereby *An. gambiae* mosquitoes exposed to ivermectin on nets and on walls sprayed with the ivermectin resulted into complete mortality in 24 h. The study also highlighted that blood feeding was affected among ivermectin exposed mosquitoes[13]. This study also pin-pointed the fact that ivermectin when applied to treated surfaces exhibited faster killing effect among mosquitoes in contrast to when ingested through blood meal from treated individuals. When feeding on ivermectin treated blood, mosquitoes survived till an average of 7 days[9,20]. Ivermectin exhibited a significantly stronger and faster mosquito-killing effect when applied to treated surfaces, eliminating mosquitoes within 24–48 h [[13]]. In contrast, when ingested through a blood meal from treated individuals, its lethal effect took an average of seven days, as reported in previous studies[9]. This variation likely arises from the limited bioavailable concentration of ivermectin in the bloodstream compared to the higher levels on directly treated surfaces[9]. Additionally, ivermectin's highly lipophilic nature may enhance its ability to penetrate the mosquito cuticle, leading to rapid paralysis and death.

In this study, we evaluated the insecticidal properties of contact-based exposure to ivermectin in *An. stephensi* and *An. culicifacies* using both bottle bioassays and topical application assays. Our data clearly shows that ivermectin is highly toxic to mosquitoes when applied topically or through contact exposure. At higher concentrations (20 µM), mortality reached 100% within 24–48 h, confirming that ivermectin contact exposure induces significant lethality in both mosquito species. The concentrations tested in the bottle bioassays ranged from 0.025 to 17.502 µg/bottle, with *An. stephensi* showing complete mortality at 17.502 µg/bottle and *An. culicifacies* showing complete mortality at 8.75 µg/bottle underscoring the increased susceptibility of *An. culicifacies* to ivermectin compared *An. stephensi*. Whereas, the topical application bioassay done within range of 0.0015 to 1.167 ng/mg for both the species. The mortality of 100%, observed at the concentration of 0.583 ng/mg and 1.167 ng/mg. These results highlighted the toxicity of ivermectin among insectary-reared mosquitoes when exposed to treated surfaces at variable concentrations. Another interesting finding of this study is that contact exposure to ivermectin has the potential to block the development of the *Plasmodium* parasite in infected mosquitoes. At a concentration of 0.010 µM, we observed ~ 71% reduction in *P. berghei* oocyst numbers, indicating that ivermectin impairs the sexual development of malaria parasite within mosquito mid-gut. Our findings contrast with a previous study wherein artificial feeding of ivermectin to *An. darlingi* did not alter oocyst intensity even in the millimolar concentration range[21]. However, we found robust inhibition of oocyst development via contact exposure at nanomolar concentrations of ivermectin, underscoring contact route as an effective mode of ivermectin delivery[13].

Given ivermectin's broad spectrum of action, including its effects on both mosquitoes and the parasite, careful monitoring and management will be essential to ensure its long-term efficacy as a vector control tool. The results of this study highlight the potential of ivermectin as a promising synergist to traditional insecticide-based vector control strategies. This transmission blocking activity adds an important dimension to ivermectin potential as a malaria control tool. The ability to reduce the transmission of *Plasmodium* directly addresses one of the key challenges of malaria control, particularly in areas where residual transmission persists despite the widespread use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS)[13].

From a medicinal chemistry perspective, these findings open new avenues for designing and exploring surface-stable, structurally related macrocyclic lactones that may exhibit improved potency and persistence for use against parasite transmission. Future studies should focus on field studies to validate these results under natural conditions, evaluate potential environmental impacts, and refine dosing strategies for optimal impact. By integrating ivermectin into existing vector control strategies, we could see a significant reduction in malaria transmission, offering hope for more effective and sustainable malaria control in India and other endemic regions worldwide. Additionally, considerations regarding the development of resistance to ivermectin in mosquito populations and the potential impact on non-target organisms must be addressed.

Data availability

All the data is included in the manuscript.

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References

1. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024
2. Pu, J. & Chung, H. New and emerging mechanisms of insecticide resistance. *Curr. Opin. Insect Sci.* **63**, 101184 (2024).
3. Raghavendra, K. et al. Insecticide resistance status of malaria vectors in the malaria endemic states of India: Implications and way forward for malaria elimination. *Heliyon* **8**(12), e11902 (2022).
4. Dev, V. et al. Malaria transmission in Tripura: disease distribution & determinants. *Indian J. Med. Res.* **142**, 12 (2015).
5. Rahi, M. et al. Malaria vector bionomics: Countrywide surveillance study on implications for malaria elimination in India. *JMIR Public Health Surveill.* **17**(10), e42050 (2024).
6. Omitola, O. O. et al. Impacts of ivermectin mass drug administration for onchocerciasis on mosquito populations of Ogun state, Nigeria. *Parasit Vectors.* **14**(1), 212 (2021).
7. Simonsen, P. E. et al. Lymphatic filariasis control in Tanzania: effect of repeated mass drug administration with ivermectin and albendazole on infection and transmission. *PLoS Negl. Trop. Dis.* **4**(6), e696 (2010).
8. Kobylinski, K. C., Tipthara, P. & Wamaket, N. Ivermectin metabolites reduce Anopheles survival. *Sci. Rep.* **13**(1), 8131 (2023).
9. Shepherd-Gorringe, M. A. M., Pettit, M. W. & Hawkes, F. M. Lethal and sublethal impacts of membrane-fed ivermectin are concentration dependent in Anopheles coluzzii. *Parasites Vectors* **17**(1), 228 (2024).
10. *Ivermectin for malaria transmission control: technical consultation meeting report*. Malaria Policy Advisory Committee, World Health Organisation. 2016. https://cdn.who.int/media/docs/default-source/malaria/mpac-documentation/mpac-sept2016-ivermectin-session9.pdf?sfvrsn=170018d7_7. Accessed on 22 April, 2025
11. Atif, M., Estrada-Mondragon, A., Nguyen, B., Lynch, J. W. & Keramidas, A. Effects of glutamate and ivermectin on single glutamate-gated chloride channels of the parasitic nematode H. contortus. *PLoS Pathog.* **13**(10), e1006663 (2017).
12. Kumar, G., Pasi, S., Yadav, C. P., Kaur, J. & Sharma, A. Potential of ivermectin as an active ingredient of the attractive toxic sugar baits against the Indian malaria vectors *Anopheles culicifacies* and *Anopheles stephensi*. *Pest Manag. Sci.* **79**(1), 474–480 (2023).
13. Hamid-Adiamoh, M. et al. Mosquitocidal effect of ivermectin-treated nettings and sprayed walls on *Anopheles gambiae s.s*. *Sci. Rep.* **14**, 12620 (2024).
14. Thakur, Y. et al. Panoramic view of diversity and function of cuticular proteins in insects and mosquitoes biology. *Front. Insect Sci.* **5**(5), 1602055 (2025).
15. Kocas, M. et al. Enhancing Intracellular Uptake of Ivermectin through Liposomal Encapsulation. *AAPS PharmSciTech* **26**, 123 (2025).
16. *Common Protocol for Uniform Evaluation of Public Health Pesticides for Use in Vector Control*, 3rd edn. Indian Council of Medical Research; Licence: CC BY-NC-SA 3.0. (2023).
17. Zheng, W. et al. Functional characterization of *Plasmodium berghei* PSOP25 during ookinete development and as a malaria transmission-blocking vaccine candidate. *Parasit. Vectors* **10**, 1–11 (2017).
18. Tenywa, F. C. et al. The development of an ivermectin-based attractive toxic sugar bait (ATSB) to target Anopheles arabiensis. *Malar. J.* **16**, 1–10 (2017).
19. Parth, S. S. et al. Antiplasmodial and insecticidal activities of third-generation ivermectin hybrids. *J. Med. Chem.* **67**(22), 20224–20241 (2024).
20. Pooda, S. H. et al. Impact of blood meals taken on ivermectin-treated livestock on survival and egg production of the malaria vector Anopheles coluzzii under laboratory conditions. *PLoS ONE* **19**(8), e0308293 (2024).
21. Kobylinski, K. C. et al. Ivermectin susceptibility, sporontocidal effect, and inhibition of time to re-feed in the Amazonian malaria vector *Anopheles darlingi*. *Malar J.* **16**, 474 (2017).

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Author contributions

MJ: writing draft, Data acquisition, Data analysis, prepared figures; BG:Data acquisition; SL: Data acquisition; GK: Data analysis, Review and editing manuscript; LKP: Data acquisition; YT: Data acquisition; GK: reviewed manuscript; VV: Data acquisition; KCP: Review manuscript; SP: Conceptualisation, Review and editing manuscript; JK: Conceptualisation, Review and editing manuscript. All authors reviewed the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to S.P. or J.K.

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