A SURVEY-BASED EVALUATION OF MOSQUITO REPELLENTS WITH REFERENCE TO THEIR SAFETY AND MECHANISM OF ACTION

A PROJECT WORK (BP812PW) SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

BY

SHAH CHARVIL (19BPH019)

VORA DARSHIT (19BPH023)

PATEL DHARMIK (19BPH030)

PRAJAPATI MITESH (19BPH077)

Semester VIII

UNDER THE GUIDANCE OF

Dr. SHITAL PANCHAL

NIRMA UNIVERSITY

INSTITUTE OF PHARMACY

NAAC ACCREDITED A+ GRADE

INSTITUTE OF PHARMACY
NIRMA UNIVERSITY
SARKHEJ-GANDHINAGAR HIGHWAY
AHMEDABAD-382481
GUJARAT, INDIA

MAY-2023

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CERTIFICATE

This is to certify that Project Work (BP812PW) entitled ***"A SURVEY-BASED EVALUATION OF MOSQUITO REPELLENTS WITH REFERENCE TO THEIR SAFETY AND MECHANISM OF ACTION"*** *is the bonafide work carried out by* ***SHAH CHARVIL (19BPH019), VORA DARSHIT (19BPH023), PATEL DHARMIK (19BPH030), PRAJAPATI MITESH (19BPH077),*** *B. Pharm semester VIII under my guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2022-2023. This work is up to my satisfaction.*

Guide:

Dr. Shital Panchal
Assistant Professor,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

Dr. Jigna Shah
Professor and Head,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University.

Prof. Tejal Mehta,
M.Pharm, Ph.D.,
Director (I/C),
Institute of Pharmacy,
Nirma University.

Date: 13 / 05 / 2023

CERTIFICATE OF SIMILARITY OF WORK

This is to undertake that the B.Pharm. Project work (BP812PW) entitled "A ***SURVEY-BASED EVALUATION OF MOSQUITO REPELLENTS WITH REFERENCE TO THEIR SAFETY AND MECHANISM OF ACTION"*** *Submitted by* ***SHAH CHARVIL (19BPH019), VORA DARSHIT (19BPH023), PATEL DHARMIK (19BPH030), PRAJAPATI MITESH (19BPH077),*** *B.Pharm. Semester VIII is a bonafide review/research work carried out by us at the Institute of Pharmacy, Nirma University under the guidance of Dr. Shital Panchal. We are aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, the review/research work carried out by us is not reported anywhere as per best of our knowledge.*

Charvil Shah(19BPH019)
Darshit Vora (19BPH023)
Dharmik Patel (19BPH030)
Mitesh Prajapati (19BPH077)

Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481, Gujarat, India

Guide:

Dr. Shital Panchal
Assistant Professor,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

Date: 13 / 05 /2023

DECLARATION

We, ***SHAH CHARVIL (19BPH019), VORA DARSHIT (19BPH023), PATEL DHARMIK (19BPH030), PRAJAPATI MITESH (19BPH077)*** *students of VIII[th] Semester of B. Pharm at the Institute of Pharmacy, Nirma University, hereby declare that our project work (BP812PW) entitled "****A SURVEY-BASED EVALUATION OF MOSQUITO REPELLENTS WITH REFERENCE TO THEIR SAFETY AND MECHANISM OF ACTION****" is a result of the culmination of our sincere efforts. We declare that the submitted project is done solely by us and to the best of our knowledge, no such work is done by any other person for the award of a degree or diploma or for any other means. We also declare that all the information was collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.*

Charvil Shah (19bph019) Charvil

Darshit Vora (19bph023) Darshit.

Dharmik Patel (19bph030) Dharmik

Mitesh Prajapati (19bph077) MP.

Institute of Pharmacy

Nirma University

Sarkhej - Gandhinagar Highway

Ahmedabad-382481

Gujarat, India

Date: 13/05/ 2023

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Shah Charvil (19BPH019)

Vora Darshit (19BPH023)

Dharmik Patel (19BPH030)

Prajapati Mitesh (19BPH077)

Institute of Pharmacy,

Nirma University

Sarkhej – Gandhinagar Highway

Ahmedabad – 382481

Gujarat, India.

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A SURVEY-BASED EVALUATION OF MOSQUITO REPELLENT WITH REFERENCE TO THEIR SAFETY AND MECHANISM OF ACTION

Shah Charvil N[1], Vora Darshit K[1], Patel Dharmik M[1], Prajapati Mitesh C[1], Panchal Shital[1*]

[1]Department of Pharmacology, Institute of Pharmacy, Nirma University, Ahmedabad-382482, Gujarat

19bph019@nirmauni.ac.in

19bph023@nirmauni.ac.in

19bph030@nirmauni.ac.in

19bph077@nirmauni.ac.in

ABSTRACT:

Since more than 60 years ago, N, N-diethyl-meta-toluamide (DEET) has been known as an extremely effective insect repellent. Even though it has been showcased to be effective in minimizing mosquito bites and reducing the risk of illnesses transmitted by mosquitoes, there are concerns about the possible adverse impacts on the well-being of humans and the environment. This study was conducted with the aim to provide an overview of the negative aspects of DEET as a mosquito repellent. This article is focused on mechanism of action, cyto-toxic effects of DEET and other ingredients and future perspective on how the insect repellents can be made safe for humans and efficacious against mosquitoes by implying some strategies that can replace harmful ingredients such as DEET with much safer and non-irritant ingredients. DEET acts on OR (Olfactory Receptors) receptor on the allosteric site to produce its effect. This article includes a survey which is conducted for utilization of different formulations of mosquito repellent and their side effects/negative impacts on participants. Survey includes various age groups from 12 to 60 that comes from rural and urban areas. Survey has been conducted using a questionnaire regarding the use of repellents and their side effects. Later, the collected data was interpreted using various data analysis techniques. The findings suggest mosquito repellents are responsible for many adverse reactions in consumers. Novel active ingredients and herbal ingredients should be explored to minimize such side effects.

1. INTRODUCTION

A product meant to repel mosquitoes and other insects is called a mosquito repellent. In order to avoid mosquito bites, mosquito repellent is often applied to the skin, garments, and perhaps other surfaces. Since a person's natural body odour is what first draws insects, mosquito repellents function by concealing this scent. On the market, there are several types of mosquito repellents, including sprays, lotions, creams, and candles are available. Majority of these products consists of an active ingredient called N, N-diethyl-meta-toluamide (DEET). Nearly, one-third of the industries produces DEET based mosquito repellents. DEET is oily compound which is slightly yellowish in color and it is applied on skin, fabrics, and other surface; used in different compositions. There are certain types of marketed formulations depending on its usage. Such are 1) Sprays like adulticides and larvicides, 2) Mosquito repellent coils, 3) Topical applicants like cream, ointments, gels, oils, etc., 4) Mats, 5) Vaporizers, 6) Fabric Roll-On, 7) TFT Papers, etc. All these different forms of mosquito repellents are manufactured and marketed by various national and multinational companies like Maxx, All Out, Good Night, Mortein, Odomos, Maxo, Mama Earth, etc. It often comprises an active ingredient that beat back mosquitoes as well as secondary substances that, among other things, dilute the active ingredient to a suitable concentration and aid in the release of the active ingredient when needed.

The general adverse effects on excessive use of this such mosquito repellents vary from person to person. It may cause allergic symptoms or contact dermatitis after using mosquito repellent. The area where the product is applied may endure swelling, irritability, and redness as a result. In addition to causing discomfort and irritation when it gets in the eyes, mosquito repellent can also cause respiratory issues, such as coughing, wheezing, and breathing difficulties in places with inadequate airflow or from inhaling excessive amounts of the repellent. There are several chemicals in insect repellents that might adversely impact the neurological system, causing headaches, vertigo, and confusion. Mosquito repellents which contain hazardous ingredients like DEET can be harmful if ingested or heavily absorbed via the skin. Based upon certain research studies, one of the most popular insect repellents, DEET, has been reported to damage cells. It interacts adversely and tends to cause the organelle responsible for energy production to malfunction i.e., the power house of cell, the mitochondria. Cellular structures may be damaged as well as oxidative stress as a result of this disruption. Also, in addition to it, human

brain cells are demonstrated to undergo apoptosis, a form of programmed cell death, because of DEET exposure.

2. MECHANISM OF ACTION

Insect repellents work by interfering with the insect's homing system. There are chemical receptors in the antennae that are used as a homing system. A natural chemical receptor is stimulated by lactic acid that evaporates naturally from warm-blooded animals' skin. The skin becomes protected by a barrier formed when repellent ingredients, such as DEET, evaporate. Consequently, the mosquito cannot bite the person because it cannot find them.

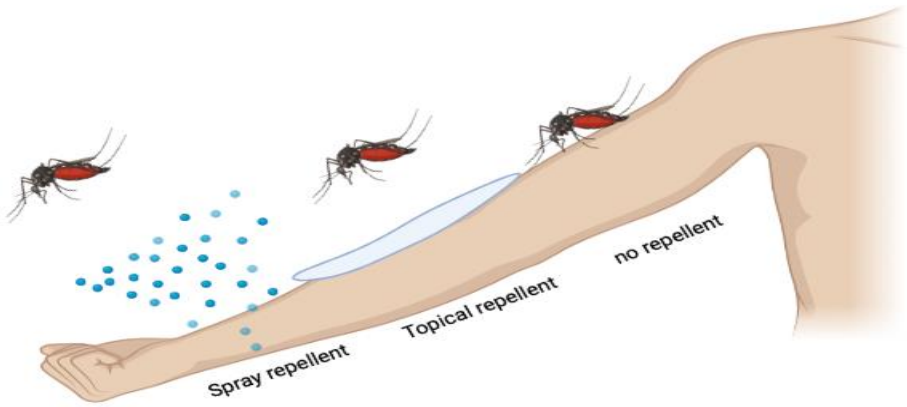

Figure 1 ***EFFECT OF TOPICAL AND SPRAY REPELLENT***

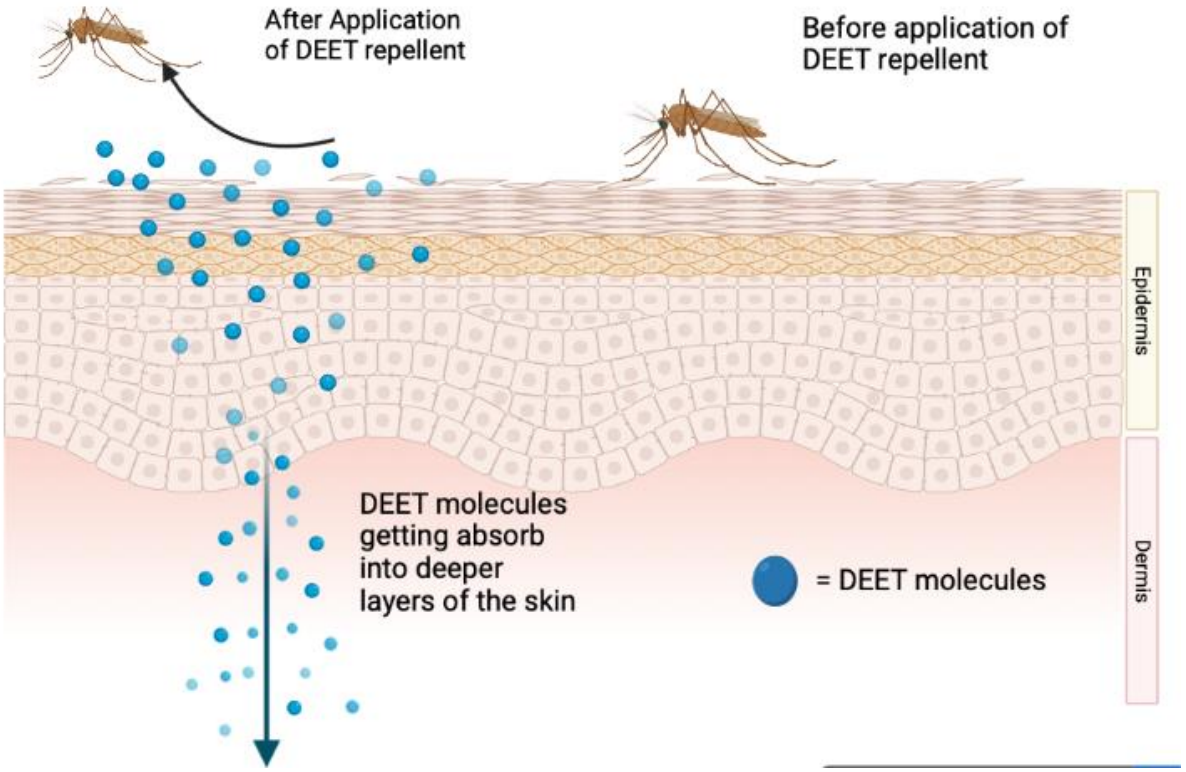

Figure 2 MOA OF DEET ON LAYERS OF SKIN

2.1 ***Action of repellents on ORNs and ORs***

Use of electrophysiological methods for examining ligand-gated ORs:

For the research of the mechanism of action of insecticides, electrophysiological recordings of ORNs in-vivo and ORs stated heterologous (in-vitro) are available. Research into the biochemical environment of ORs is currently ongoing because we only have a fundamental understanding of the components. Insect ORs are heterogeneous ligand-gated ion channels. They are composed of the variable odorant-sensing subunit (ORx), also known as ORx-Orco, and the obligatory and constant OR coreceptor (ORCO)[1-3]. While the odorant receptors (ORs) are essential for olfactory signalling, odorant binding proteins (OBPs), sensing neuron membrane proteins (SNMPs), and odorant degrading enzymes (ODEs) may also have an impact on OR action.

ORs can be produced using a variety of those cells which has expressing system, such as the oocytes found in the frog Xenopus laevis, which are not in the sensillum, to get rid of these influences. The amount of the stimuli in which the receptors undergo stimulation is known, allowing this pharmacological method to create exact concentration-response connections. It is of the utmost importance to have prior understanding about the OR and its naturally occurring ligand. Resembling categories of receptors, ORs are likely to have numerous recognising sites, involving a putative primary "orthostatic" site that interacts with chemical compounds chosen by development and secondary "allosteric" sites that respond to chemical substances without a clear biological definition (such as synthetic compounds)[4].

On basiconic sensilla, somewhat related tests can be performed in vivo, which contain triple ORNs that can be operationally identified by looking at the form and intensity of their action potentials, in order to assess the pharmacological knowledge gained using Xenopus oocytes. CO2 triggers a response from the "A" neuron, which has the biggest amplitude action potential[5, 6]. The "B" neuron produces moderate action potentials on a fundamental basis with no known effective stimulation. The "C" neuron, which has the shortest action potential amplitude, is assumed to possess the OR8-Orco receptor assembly because it reacts to octenol[7]. There is one notable restriction on the presence of dose-response correlations between ORN activity and odorants or medicines, even though single-cell documentation is an effective method for studying neuron physiology in vivo: it is not possible to determine the precise amount of stimulus that leaves the odour cartridge and reaches the ORN's outer layer.

In 1989, octenol (=1-octen-3-ol) was shown to be naturally derived ingredient. The "C" neuron, situated on the maxillary palps in the basiconic sensilla, has been shown in a study employing single-cell observations in Culex pipiens to be selective towards the (R)-enantiomer of octenol[8, 9]. In the same year, two power clamping experiments were performed on Xenopus oocytes expressing the An gambiae octenol receptor (OR8-Orco), and the results revealed improved responses to the (R)-enantiomer of octenol[10]. The Ae aegypti OR8 ortholog was used for a complete pharmacological investigation, and the results suggested that (R)-octenol was the main odorant ligand for OR8 in relation to expression levels[11].

The ORNs' and ORs' odorous substance responsive range.

The responses of the OR8-Orco to exposure to octenol and its several structurally unchanged counterparts were studied using voltage clamp measurements. Given that each sample that was examined had traces of the opposite enantiomer, it is likely that the (R) enantiomer's response to OR8-Orco was overstated by about 100 times compared to the (S) form[11]. Perhaps even more striking was the fact that OR8-Orco affinity was stronger for various octenol analogues than for (S)-octenol. The results of this study demonstrated that an OR could accurately and sensitively identify a non-pheromonal molecule.

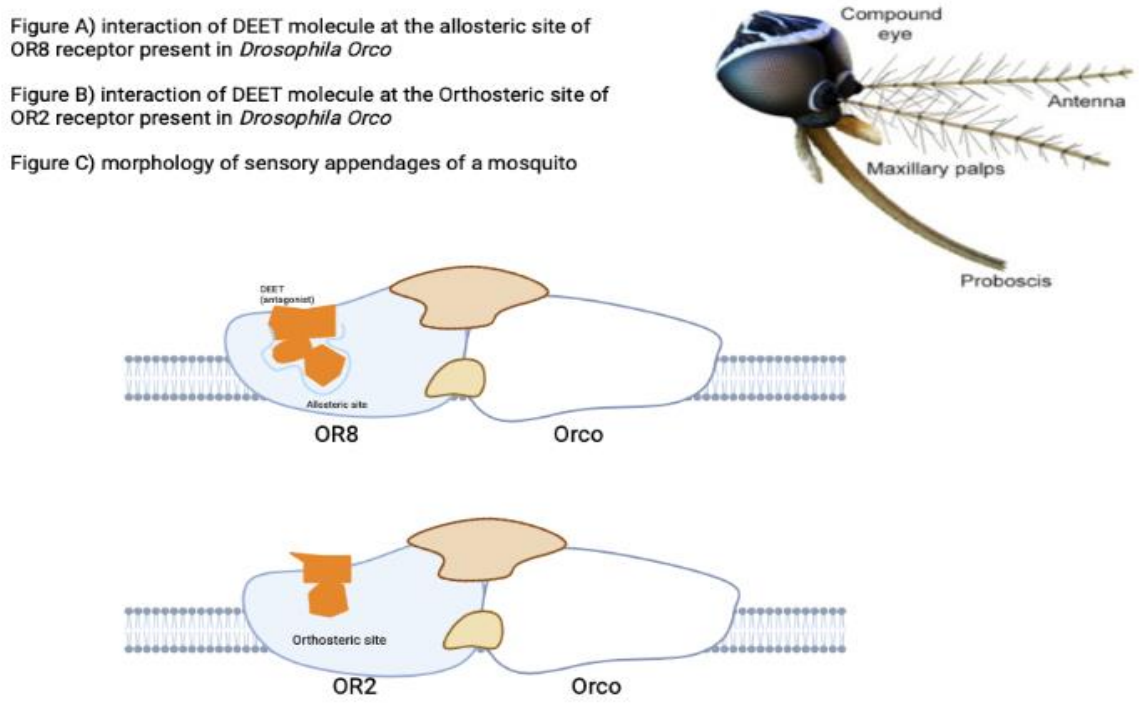

Figure 3 ***ACTION OF REPELLENT ON ORNs AND ORs***

Utilising only one cell observations compared to octenol-sensitive ORNs in sensilla on the maxillary palps, the outcomes of the *ex vivo* investigations were validated in living organisms. The identical octenol analogues and each of the octenol enantiomers evaluated in the heterologous expression system were applied to the "C" neuron while it was operating in carbon dioxide (CO2) pure air. Even though it was impossible to precisely calculate the amount of odorant reaching the ORN, the results were consistent with those obtained from voltage clamp measurements made from Xenopus oocytes expressing OR8-Orco. The greater fluctuations in

the sensitivity to (R)-octenol compared to other compounds that were detected *in vivo* were difficult to interpret because of the inherent differences in stimulus delivery between voltage clamp and single cell recording techniques. A role for accessory proteins like OBPs in enhancing the sensitivity of OR8-Orco in vivo could not be fully ruled out given that various studies have indicated that OBPs may change the solubility of smells in the sensillum fluid enclosing the neuron.

Two more ORs offered pharmacological opportunities to investigate the impact of insect repellents. The paralogous indole receptors OR2 and OR10 are found in both aedine and anopheline mosquitoes and are part of a preserved set of indole receptors. With the help of voltage clamp observations from Xenopus oocytes generating OR2-Orco, the physiologically significant ligand for OR2 indole was discovered. It was anticipated that because OR2 and OR10's proteins have the same sequence, the second one would particularly recognise an indole analogue. Later, it was established that 3-methyl-indole (skatole) is the cognate ligand for OR10. Three significant findings emerged from these experiments: (1) Without the help of other variables like OBPs, ORs have the ability of a surprising level of discriminating, (3) The molecular shape of chemical compounds is a determining factor for appropriate ligand identification, and (2) The ligand responsiveness and specificity of non-pheromone receptors are comparable to those of pheromone receptors.

The previously mentioned research' findings supported the hypothesis that ORs have an extremely specialised primary identification site (orthostatic site) for a physiologically significant signal (semi chemical), and "specialist" ORs now comprised non-pheromonal receptors. These concepts necessitated a reconsideration of the notion of "generalist" ORs, or receptors that are triggered by ligands with different chemical geometries. Only one yet variable-geometry ligand identification site (individual binding domain) or several detection locations (multiple binding domains) are both necessary for an OR to be able to recognise a variety of ligands. The latter theory is being supported by increasing experimental proof, albeit some restricted ligand promiscuous is still possible[11].

DEET modulates the activity of ORs:

DEET, a well-known insect repellent, has been found in different experiments to selectively trigger or block ORs. Even at high quantities, this artificial substance, which flies had never met in nature, was able to communicate with ORs. How may this substance have opposing agonistic and antagonistic actions on various ORs? We investigated this question using our understanding of OR-ligand pairings. The sequences of OR2, OR8, and OR10 share a variety of commonalities. The amino acid identities of OR2 and OR10 are 69% and 14%, respectively, those of OR8. These changes in protein sequence served as a control to examine the molecular underpinnings of the potential method of action of insect repellents. Additionally, using Xenopus oocytes, we broadened our experiments to include insect repellents with various structural differences, comprising both artificial and naturally produced molecules.

These investigations showed that repellents for mosquitoes had a variety of impacts on ORs. DEET, for instance, stimulated OR2-Orco when used alone, but also restricted OR8-Orco's reaction to octenol. DEET decreased OR sensitivity and the agonist odorants' maximal effect[12]. A review of the potential impacts of several insect repellents on OR8-Orco and OR2-Orco revealed that, depending on the ORx examined, these substances either triggered specific, dominant, or unspecific agonist or antagonist actions. More crucially, in vivo investigations that demonstrated how DEET affected the fine-tuning of functionally different ORNs substantially supported these findings. The multimeric structure of ORs complicated the interpretation of these studies, making it difficult to pinpoint the precise processes by which insect repellents work via orthostatic or allosteric sites.

In order to find new regulators of An. gambiae ORs expression in human embryonic kidney cell lines, high-throughput screening was used. It was discovered that a new class of artificial substances known as VUAA can activate Orco on its own[12]. Since Orco is the non-sensing component of the ORx-Orco complex, the findings we obtained served as evidence of theory for the presence of allosteric sites. Another investigation into how a VUAA analogue activates Orco was in line with the theory that Orco generates multimeric assemblages[13].

2.2 Action of repellents on GRNs and GRs:

While much research on mosquito repellents has focused on the olfactory sense, it has been suggested in a few papers that repellents may also be recognised by gustatory receptors (=contact chemoreceptors) on the labella and operate as feeding inhibitors. Low-volatile chemicals, according to Christophers, are surface repellents. Bar-Zeev and Schmidt presented findings indicating that the contact-sensitive chemical receptors on the labella of Ae. aegypti could detect low doses of DEET using a radiotracer. DEET as well as other repellents have recently been proven to prevent feeding in behavioural bioassays[14, 15]. It's possible that insect repellents target gustatory receptors given the theory that DEET and various other repellents served as feeding inhibitors.

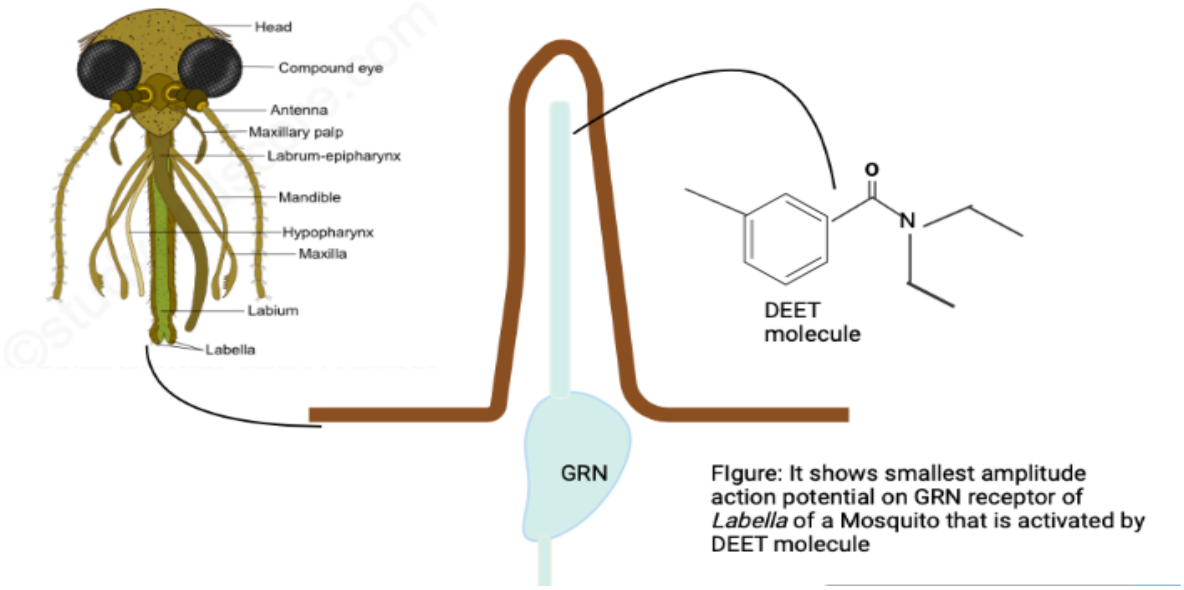

Figure 4 ACTION OF REPELLENT ON GRNs AND GRs

We newly showed the existence of a GRN that reacts to DEET and other repellents like Picaridin, IR3535, and citronellal using just one cell observations from sensilla on the labella of Ae. aegypti. A minimum of three GRNs are stored within distinct sensilla on the labella,

depending on their size and form of the action potentials that were seen. Raising NaCl concentrations elicited a response from the neuron having the highest intensity action potential. Sucrose induced an action potential with a somewhat lesser amplitude, while quinine, a feeding inhibitor, activated the lowest amplitude action potential. DEET additionally activated this neuron, which had the lowest action potential intensity, in a dose-dependent manner, while responses were induced by picaridin, IR3535, and citronellal. GRNs which is present in the fruit fly, D. melanogaster can also be activate by DEET.

It is a first for mosquitoes or indeed any hematophagous arthropod to find a GRN which reacts consistently to DEET as well as different repellents. This preventive GRN is like GRNs that mediate defensive behaviours in herbivorous insects. Given that GRNs from different insects, such as the blowfly Phormia regina, react to volatile substances at elevated concentrations of substances, the neuron's sensitivity to the examined repellents may offer, within part, another explanation for the need for these repellents to be used at extremely high levels. Since citronellal is structurally distinct from DEET, Picaridin, and IR3535 and is a synthetic chemical, it is possible that certain repellents communicate with allosteric sites on the GR assemblage, as has been more clearly shown for the olfactory sense[16].

3. MECHANISM OF ACTION OF SIDE EFFECTS OF DEET

Uncertainty surrounds the precise methods by which DEET (N, N-Diethyl-Meta-Toluamide) adversely affects human skin. However, several theories have been put out to explain how it can have negative effects. According to one theory, DEET has the ability to pass through the skin and into the bloodstream, where it may interfere with the nervous system’s ability to function normally. As a result, you can symptoms like headache, lightheadedness, or seizures, which have been linked to DEET exposure in some situations.

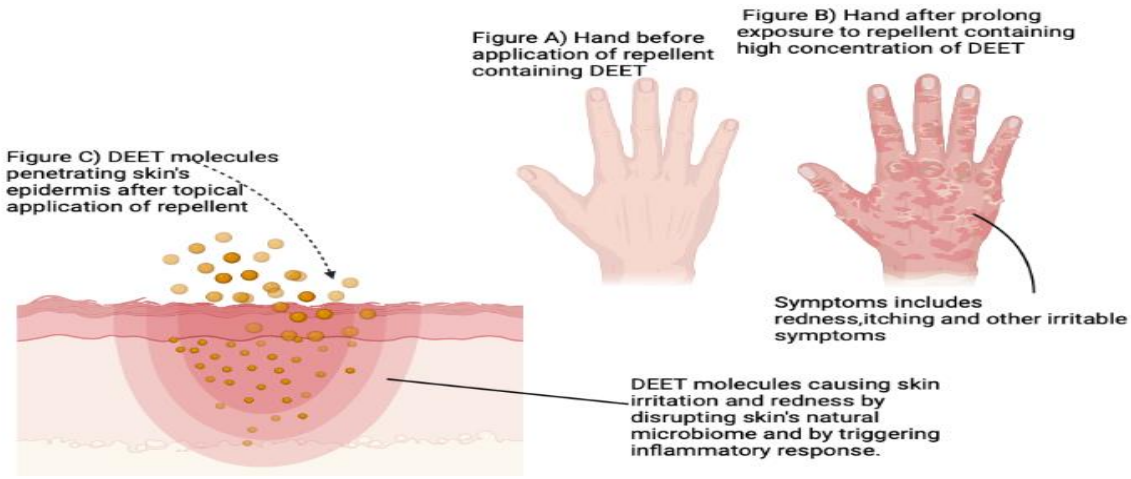

Figure 5 MOA OF SIDE EFFECT OF DEET ON SKIN

According to a different idea, DEET may irritate or trigger allergic reactions on the skin by upsetting the microbiome, or community of bacteria and other microorganism that reside on the skin's surface. DEET has the potential to irritate the skin, causing irritation-related symptoms as redness, itching, and swelling. DEET can also interact with some textiles and polymers, melting or dissolving them. Clothing, eyeglasses, and other items that come into contact with skin or clothing that has been treated with DEET may get harmed as a result. The concentration of the substance used, the length of exposure, and the person's susceptibility to the chemical all affect harm severe the adverse effect of DEET. It is crucial to use DEET based insect repellents in accordance with the direction on the packaging as well as minimizing the unnecessary utilization or extended contact to the chemical, in order to reduce danger of side effects. It is advised to stop using the product and clean the area of concern with soap and water if skin irritation or other complications appear.

4. GENERAL SIDE EFFECTS

People frequently use insect repellents that include DEET. DEET ought to be used with precaution as it may break down plastic and vinyl (such as eyeglass frames) and ruin spandex, rayon, acetate, and colored leather[17]. DEET cannot be used in mosquito nets or in many urban areas since it harms painted and lacquered surfaces as well as synthetic materials[18, 19]. The risk assessment of DEET has received extensive research because it is the benchmark

of repellents. When DEET is placed to the skin at the recommended dose on a commercially available product (and not absorbed or massaged into the mucous membranes), approximately 15 million individuals in the UK, 78 million people in the USA, and 200 million people worldwide do so safely every year. Since its introduction in 1946, DEET has been utilized without many known adverse reactions, many of which were caused by excessive or incorrect usage of repellent. Its toxicity has received more attention than that of any other mosquito repellent, and it has been found to be harmless for use on people, including children and pregnant women and lactating women[20, 21].

4.1 Side effects in adults

Case reports of DEET consumption are the primary source of information on the chemical's toxicity to humans. Within an hour of ingestion such a substance, convulsions, hypotension, and coma may occur[22]. Serum levels of 1 mmol/L have been responsible for fatalities[23]. It is not known what causes seizures. They could show up as quickly as an hour after consumption or up to 48 hours later[23]. No interactions have been verified; however, seizures may theoretically happen more frequently in DEET users who are also taking medications that reduce the seizure threshold such as bupropion, antipsychotic, systematic steroids, and antimalarials. A mature person who had utilized a product with 70% DEET to their skin was reported as having psychosis[23]. Following cutaneous application, immediate contact dermatitis, generalized pruritus, and generalized angioderma have all been reported[23]. Absorption to the conjunctiva may be use conjunctival injury.

4.2 Side effects in children

Seizures might be a significant negative effect in children based on extrapolating information on toxic consequences in adults. Nevertheless, in the nearly 50 years since DEET became accessible, we only discovered 10 reports showing seizure in kids after dermal application of DEET; none were published after 1992[24-30]. Despite this, such instances case has received a lot of attention and have promoted pediatric societies and regulatory bodies to restrict the consumption of DEET in young infants. However, given that seizure disorders affect 3% - 5% of kids and that 23% - 29% of children on this continent are thought to have been exposed to DEET, it would not be unusual to see a link that occasionally results from pure chance[30, 31]. Case studies are not helpful in establishing causation when two events (such as DEET exposure and seizures) are both common, according to epidemiology. Additionally, because other characteristics of those reported cases do not seem to be pathogenic, they are not very helpful.

In none of the instances, for instance, was encephalitis caused by viruses ruled out. In cases, encephalitis was considered as a possible multiple diagnosis, while “nonspecific rash” was described in 1 case. Reye’s syndrome, which by itself might lead a kid to seizures, was reminiscent of the clinical scenario in another instance[24, 32]. After examining 5 pediatric occurrences of seizures linked to topical administration of DEET in the end of 1989, the CDC issued a warning against using these cases as evidence of causality. To be more precise, the CDC stated that,"DEET ought not to be considered as the root cause of an epileptic seizure unless a suitable test has firmly eliminated another probable aetiology”

The connection has been inferred by authorities’ multiple times, but no proper review has bee n done in any event prior to or after 1989. Depending on information gathered by the American Association of Poison Control Centers during the year 1993 and the year 1997, a sizable based on populations study on the health effects of DEET was released in 2002[33].

The authors gathered information on instances of unintentional DEET exposure in children an d adults, and then they analysed the cases according to chronological age and the degree of ne gative effects (which ranged from none to fatal). The long-held, unsupported belief that youngsters tend to be more susceptible to the negative effects of DEET than adulthood is disproved by this research.

4.3 Side effects in Pregnant and Lactating Women

Women frequently worry about utilising DEET while pregnant, but the information that curre ntly exists on the harmful consequences in humans as well as animals is promising. A recent investigation using animals found no negative effects, with a single exception, in the progeny of rabbits and rats that were forced DEET at various dosages and gestational ages. The greatest DEET dose, which was several orders in the range greater than the typical huma n dose (325 mg/kg daily), caused harmful reactions in the mother and caused birth weights th at were small in the children[34]. A randomized, double bling investigation including 897 pregnant women in the nation of Thailand who constantly applied therapeutic concentration of DEET topically (1.7 g/d), an amount identical to that advised for minimizing malaria, or a placebo to block malaria, served as a preliminary study of its safety of DEET when used during both the second and third trimester of pregnancy[35]. The median total dose given to the DEET group was 214.2 g. A segment of 50 women had their cord blood samples tested for DEET amounts; the substance was found in 4 (or 8% of them), proving that it passing the placenta[36]. None of the women subjected to DEET in the group of participants showed any negative neur

ological, gastrointestinal, or dermatological symptoms and no negative impacts on the babies' staying alive or ability to grow and develop at delivery or at twelve months of age were identified[37].No research on DEET exposure during the first trimester in humans was found. The highly concentrated oral dose employed in the animal study. However, demonstrates that DEET is tolerable when taken as advised[34].

Neem's safety has been well investigated, and azadirachtin is not poisonous to mammals and does not exhibit chronic toxicity. Neem products did not cause skin irritations, organic changes, or mutagenic or carcinogenic effects in mice or rats, even at elevated doses. On the contrary, daily application of an aqueous extract of leaves to rats for 6 and 9 weeks caused irreversible reproduction problems that resulted in sterility in the rats at 66.7 and 100%, accordingly. If used carefully, the use of aqueous, uncooked neem-based products should be promoted. In contrast to nonaqueous extracts, that turn out to be comparatively poisonous, the pure chemical azadirachtin, uncooked materials, watery extracts, and seed oil are harmless to utilize even as insecticides to preserve preserved food for humans to eat. Azadirachtin is harmless and harmless to fish, natural enemies, pollinators, birds, and other wildlife from an ecological and environmental perspective. Azadirachtin is categorized by the US EPA as class IV (practically harmless) since it degrades in water within 50–100 hours and in sunlight because its half-life is only one day, leaving no leftovers.

5. ALTERNATIVES OF DEET

Other insect repellents besides those containing DEET is offered in Canada, but there is not much information on their safety. Citronella oil topical products come in concentrations ranging from 5% to 15%. There are also burning candles and burning incense made with citronella. Although a recent study found that the average protection period was shorter than twenty minutes with a substance containing 10% levels[38], the duration of defense time with dermally applied treatments is generally thought to be anywhere from thirty minutes and 2 hours. 6% lavender oil applied topically provides protection for a maximum of thirty minutes[30, 39]. Despite the fact that citronella and lavender oils are usually regarded as being risk-free, it is not advised to use them topically to children under the age of two due to a lack of data. Ingestion of any oil may raise concerns about aspiration pneumonia.

A product with only two percent soybean oil in it was discovered to provide protection for approximately ninety minutes, which is comparable to the time provided by a product with 4.75% DEET[38]. But DEET at 6.65% and 20% concentrations provide protection for an average of 120 and 210 minutes, accordingly. Although there have been authorized soybean oil-based repellents in Canada,

none yet exist on the market. If just a kid consumes soybean oil, aspiration pneumonia may be a problem.

In one field experiment, a novel item called OFF! Botanical products Topical Mosquito Repellent 1 that contains 10% p-menthane-3,8-diol was discovered to be efficacious for a minimum of ninety minutes[30]. The age restriction only reflects a paucity of data; for children older than 3 years old, it may be given up to two times per day[30, 40]. There are not any epidemiological reports of the substance's safety following cutaneous contact or oral administration, despite the manufacturer's claim that no side effects are anticipated with proper use.

The latter unable to demonstrate sufficient defense against attacks by mosquitoes in controlled research that contrasted repellents with and without DEET[38].

6. AIM AND OBJECTIVES:

This study presents survey based the evaluation and discussion on mosquito repellents and their side effects on human skin and cells. The most used mosquito repellent is DEET and this article focuses particularly on this ingredient. It also includes effects of DEET on mosquito as a mosquito repellent their mechanism of action and DEET's cytotoxic effects on human skin as a topical cream, aerosol spray, electric coil, vaporizer, and mats etc. The basic objective of the study was to conduct survey to determine use of mosquito repellent and their side effects.

7. STUDY METHOD:

The individuals aged from 12 or olde who uses mosquito repellent are localized from rural and urban areas were involved in the survey.150 participants had responded to google form. Sample size included 123 individuals from urban area and 27 from rural area. The duration in which the survey has been conducted is approximately 3-4 months. The study is based on general questionnaire and interviews and study includes open-ended and close-ended questions. These questions were about the utilization of insect repellents and side effects or negative impacts on participants which they may have experienced were included in general questionnaires and interview. When asked, it was discovered that the majority of people didn't know how to use repellents and only used them because they had seen an advertisement or because a friend or member of their family had advised them to. The questionnaire is attached here as appendix-1. The collected data was analyzed using Microsoft excel and statistical test that were used

includes T-test: paired two sample for means (Microsoft excel) and paired parametric T-test (GraphPad prism). In T-test, t-statistic value is less than t-critical value than it considered to be significant and P value is less than 0.05 than data is significantly effective.

8. RESULTS:

Table 1: Age Distribution of Participants

Age group in years	Semi-rural area (N=27) (18%)	Urban area (N=123) (82%)	Total (N=150) (100%)
12-24	23 (85.18%)	92 (74.79%)	115 (76.66%)
24-40	3 (11.11%)	12 (9.75%)	15 (10%)
40-60	1 (3.70%)	16 (13%)	17 (11.33%)
60+	0 (0%)	3 (2.24%)	3 (2%)

According to the survey conducted, out of 100%, 76.66% were from 12 to 24 age group. Remaining 23.33% were from the age group above 25 years. The survey also justified many participants were from urban areas that was 123 out of 150 participants and merely 27 participants belonged from semi-rural or rural area. (Table 1, Figure 6)

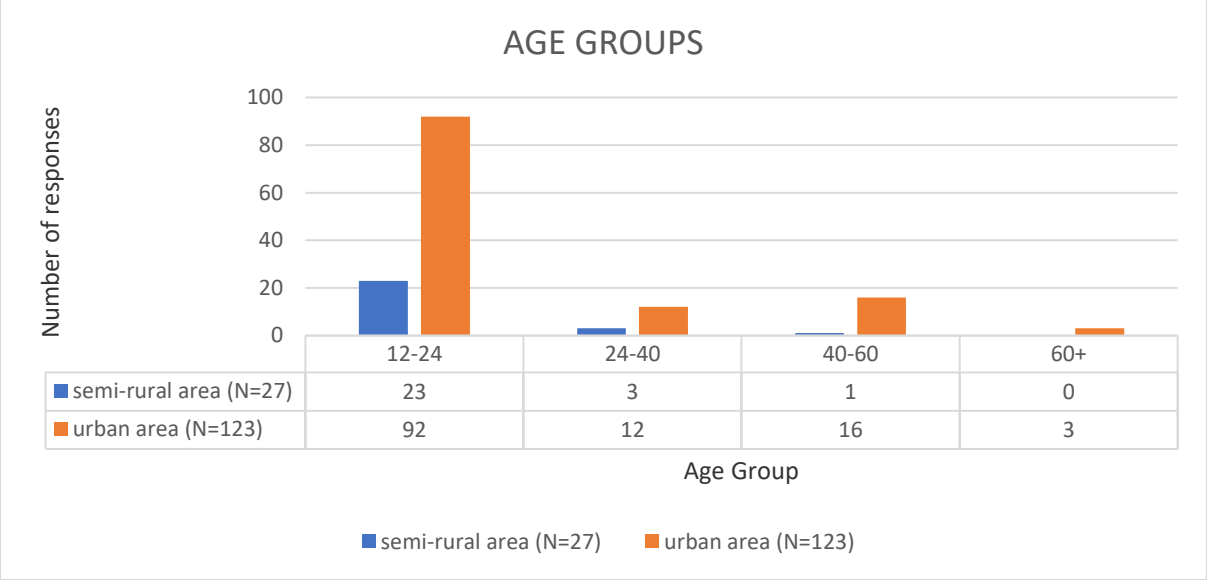

Figure 6 Age Distribution of Participants

Table 2: Educational Background of Participants

Education	**Semi-rural area (N=27)**	**Urban area (N=123)**	**Total (N=150) (100%)**
10th pass	1 (3.70%)	5 (4.06%)	6 (4%)
12th pass	4 (14.80%)	13 (10.56%)	17 (11.33%)
UG program	18 (66.66%)	85 (69.10%)	103 (68.66%)
PG program	3 (11.11%)	17 (13.82)	20 (13.33%)
PhD	1 (3.70%)	3 (2.43%)	4 (2.66%)

In accordance with the further questions in survey, the academic background of the participants differs proportionally with urban and semi-rural areas. Here participants from UG program dominates the survey in both urban (69.10%) and semi-rural area (66.66%), with the total of 103 participants out of 150 which delivers 68.66%. Followed by PG programs at 13.33%; 12th pass at 11.33% and least for PhD 2.66%. (Table 2, Figure 7)

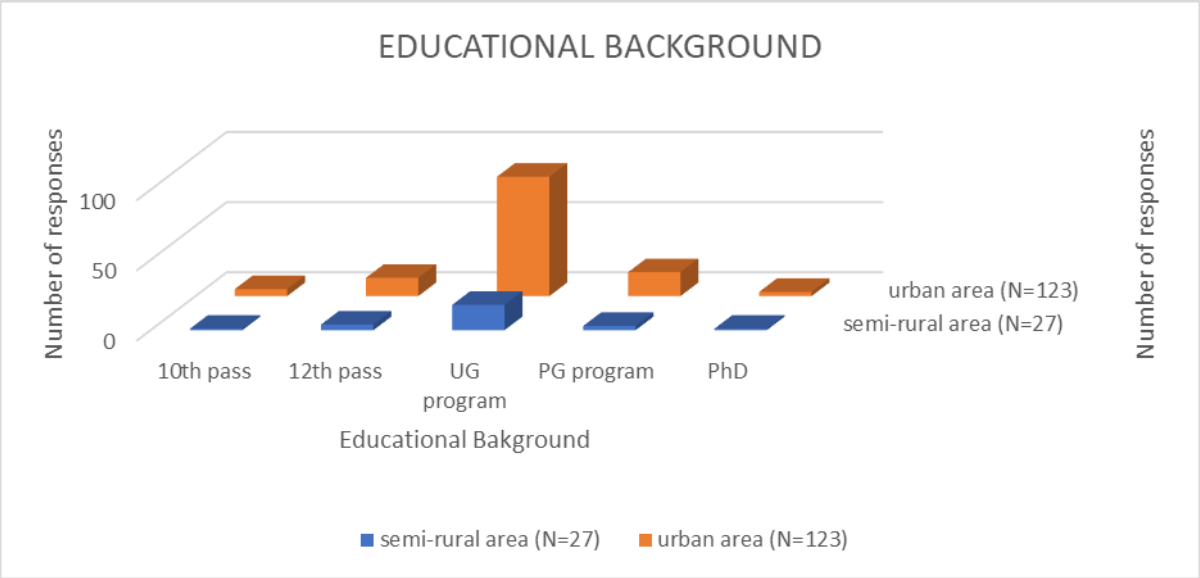

Figure 7 Educational Background of Participants

Number of Participants using Mosquito Repellent

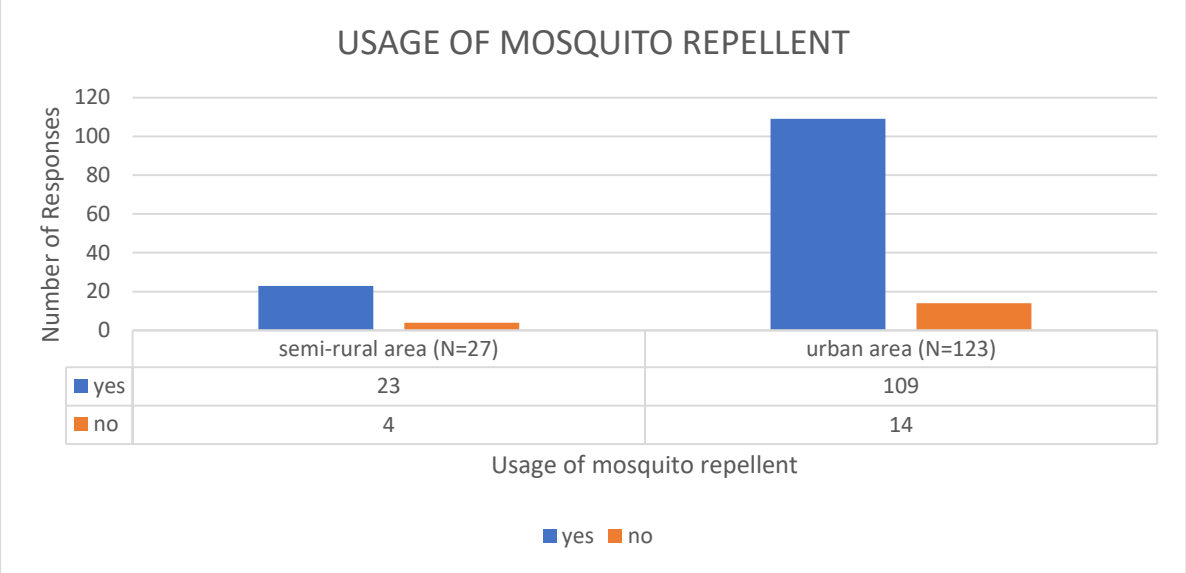

Figure 8 ***Number of Participants using Mosquito Repellent***

From urban and semi-rural areas, approximately 90% population are using mosquito repellent products from hindering the mosquito biting. (Figure 8)

Table 3: statistical analysis of usage of mosquito repellents.

	T-STATISTICS (T)	**P-VALUE (T<=t)**	**T-CRITICAL (t)**
One tail test	1.5	0.1871	6.3137
Two tail tests	1.5	0.3743	12.7062

This table: 3 of the t-test: Paired Two sample for Mean analysis shows that Y-statistics (T) is less then T-critical (t) value, so our assumption illustrated that there is no difference in yes and no response of uses mosquito repellent in urban and rural areas in different forms. (Table 3)

Table 4: Duration of Use of Mosquito Repellent

Duration of Usage	Semi-rural area (N=27)	Urban area (N=123)
once a day	11 (40.74%)	58 (47.15%)
seasonal	11 (40.74%)	41 (33.33%)
twice a day	4 (14.81%)	14 (11.38%)
whole day	1 (3.70%)	10 (8.13%)

This given table describes the data of time duration of mosquito repellent in urban and semi-rural area. It gives a clear idea that urban volunteers use the maximum repellent products. From which 58 participants use it once a day; followed by seasonal (41) and twice a day (14); least (10) for whole day. Compared to urban people, semi-rural community use less repellent product. (Table 4, Figure 9)

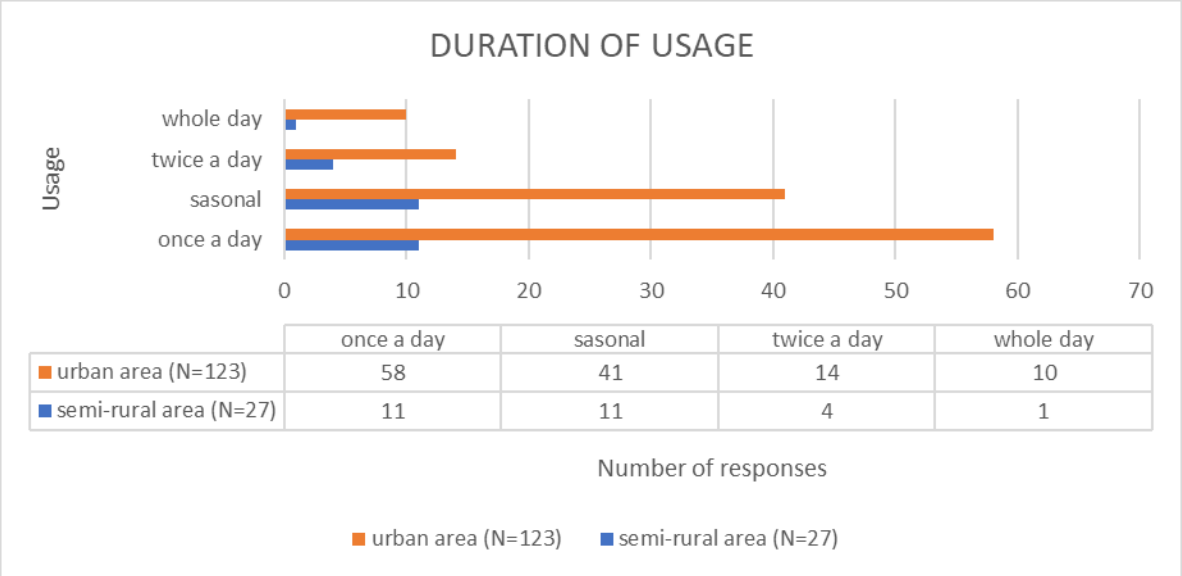

Figure 9 Duration of Use of Mosquito Repellent

Table 5: statistical analysis of data of duration of usage of mosquito repellents

Paired T-test	P-value (P<0.05)	Significantly effective
One tailed	0.0305	Yes

From statistical analysis, observed P value was 0.0305 i.e., less than 0.05 so there is no significant difference available between duration of use of mosquito repellents in urban and semi-rural areas. (Table 5)

Table 6: Types of Formulation

Type of formulation	Semi-rural area (N=27)	Urban area (N=123)
cream/ointment/gel	13 (48.14%)	76 (61.78%)
electric coil	13 (48.14%)	63 (51.21%)
aerosol	6 (22.22%)	19 (15.44%)
normal coil	5 (18.51)	22 (17.88%)
fabric roll on	8 (29.62%)	16 (13%)
other	0 (0%)	3 (2.43%)

This data shows that majority of population uses topical formulation like cream/ointments/gel in urban (61.78%) and semi-rural (48.14%); which is leaded by electric coils in urban (51.21%) and semi-rural (48.14%). Further this data is followed by aerosol, normal coil, fabric roll-on etc. (Table 6, Figure 10)

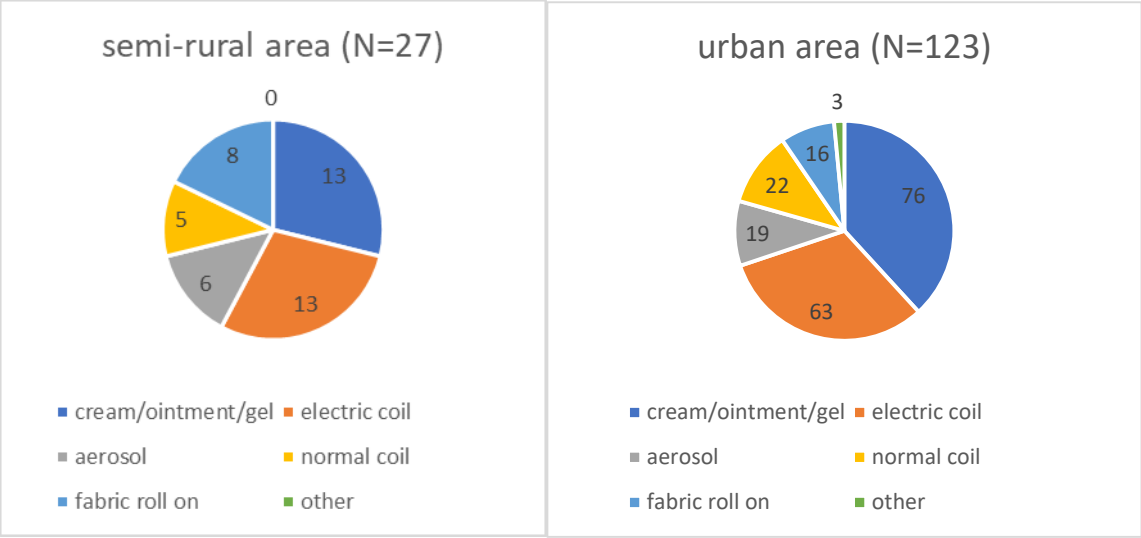

***Figure 10* Types of Formulation**

Table 7: statistical analysis of data of types of formulations of mosquito repellent

Paired T-test	P-value (P<0.05)	Significantly effective
One tailed test	0.0056	Yes

From statistical analysis, observed P value was 0.0056 i.e., less than 0.05 so there is no significant difference available between types of formulation of mosquito repellents used in urban and semi-rural areas. (Table 7)

Table 8: Types of Brands of Mosquito Repellents Used by Participants

Brand name of repellent	Semi-rural area (N=27)	Urban area (N=123)
All out by SC Johnson	8 (29.2%)	66 (53.65%)
Goodnight by Godrej	15 (55.55%)	58 (47.15%)
Mortein	17 (62.96%)	67 (54.47%)
Odomos cream	17 (62.96%)	48 (39.02%)
Maxo	5 (18.51%)	17 (13.82%)
other	1 (3.70%)	3 (2.43%)

Above date describes, the majority of crowd uses Mortein in urban (54.47%) and for semi-rural (62.96%). Followed by All Out by SC Johnson (53.65% and 29.2%), Goodnight by Godrej (47.15% and 55.55%), etc. (Table 8, Figure 11)

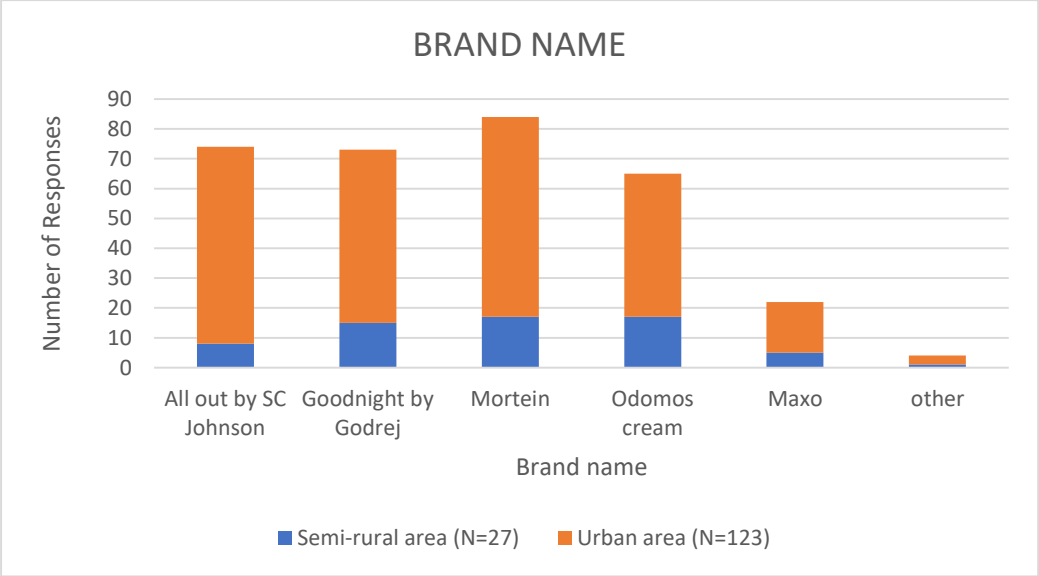

Figure 11 ***Types of Brands of Mosquito Repellents Used by Participants***

Table 9: statistical analysis of data of types of brands of mosquito repellents

Paired T-test	**P-value (P<0.05)**	**Significantly effective**
One tailed test	0.0316	Yes

From statistical analysis, observed P value was 0.0316 i.e., less than 0.05 so there is no significant difference available between types of brands of mosquito repellents used in urban and semi-rural areas. (Table 9)

Table 10: Awareness in Participants About Ingredients of Mosquito Repellent

Have you observed ingredient behind package labelled	**semi-rural area (N=27)**	**urban area (N=123)**
yes	14 (51.85%)	65 (52.84%)
no	7 (25.92%)	33 (26.82%)
maybe	6 (22.22%)	25 (20.32%)

Hence, we observed that, majority of the populations read the active ingredients listed at back label of the product. In urban area 52.84% and in case of semi-rural area 51.85% participants used to read the content of formulation. Whereas, remaining are not much interested to observe the label or some observe sometimes. (Table 10, Figure 12)

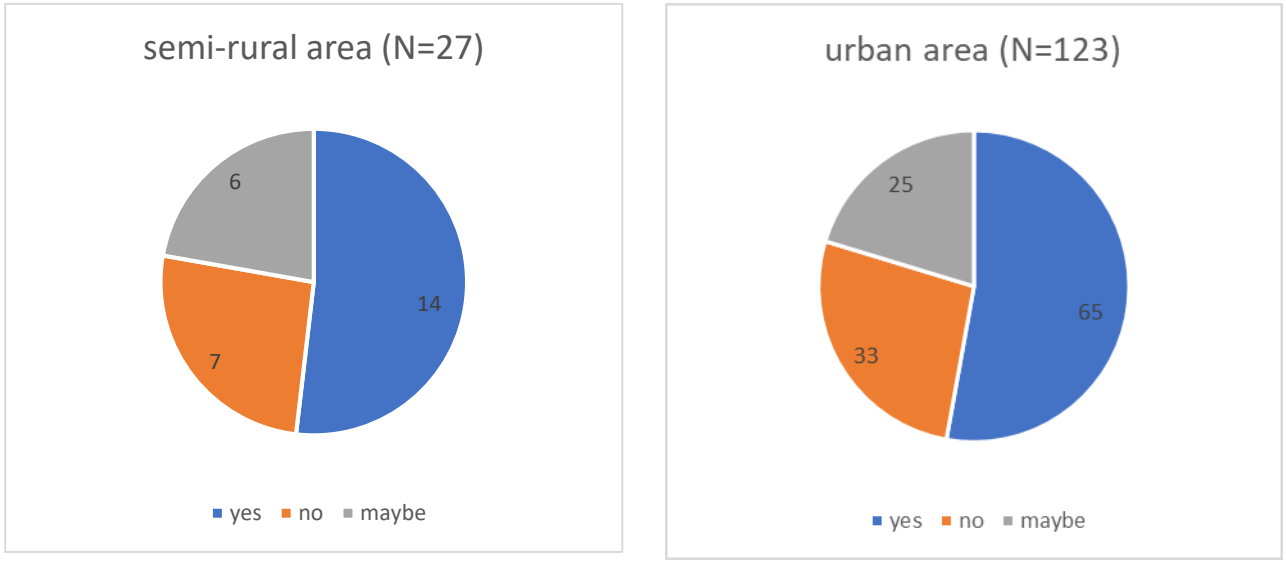

Figure 12 ***Awareness in Participants About Ingredients of Mosquito Repellent***

Table 11: statistical analysis of data of awareness of participants about ingredient of mosquito repellents

Paired T-test	**P-value (P<0.05)**	**Significantly effective**
One tailed test	0.0239	Yes

From statistical analysis, observed P value was 0.0239 i.e., less than 0.05 so there is no significant difference available between awareness in participants about ingredients of mosquito repellents used in urban and semi-rural areas. (Table 11)

Table 12: Types of Ingredients in Mosquito Repellent

which ingredient	**semi-rural area (N=27)**	**urban area (N=123)**
DEET	13 (48.14%)	55 (44.71%)
Para menthane 3,8 diole	4 (14.81%)	14 (11.38%)
citronella oil	4 (14.81%)	11 (8.94%)
neem oil	15 (55.55%)	39 (31.70%)
transfluthrin	10 (37.03%)	18 (14.63%)
not applicable/not remember	10 (37.03%)	39 (31.70%)

According to the data, in urban and semi-rural area 44.71% observed DEET as an active ingredient and 48.14% respectively. Followed by NEEM oil, 31.70% and 55.55% respectively; Transfluthrin for 14.63% and 37.03% respectively. Para-manthan-3,8-diole contributes 11.38% and 14,81% respectively; Citronella oil 8.94% and 14.81% respectively. (Table 12, Figure 13)

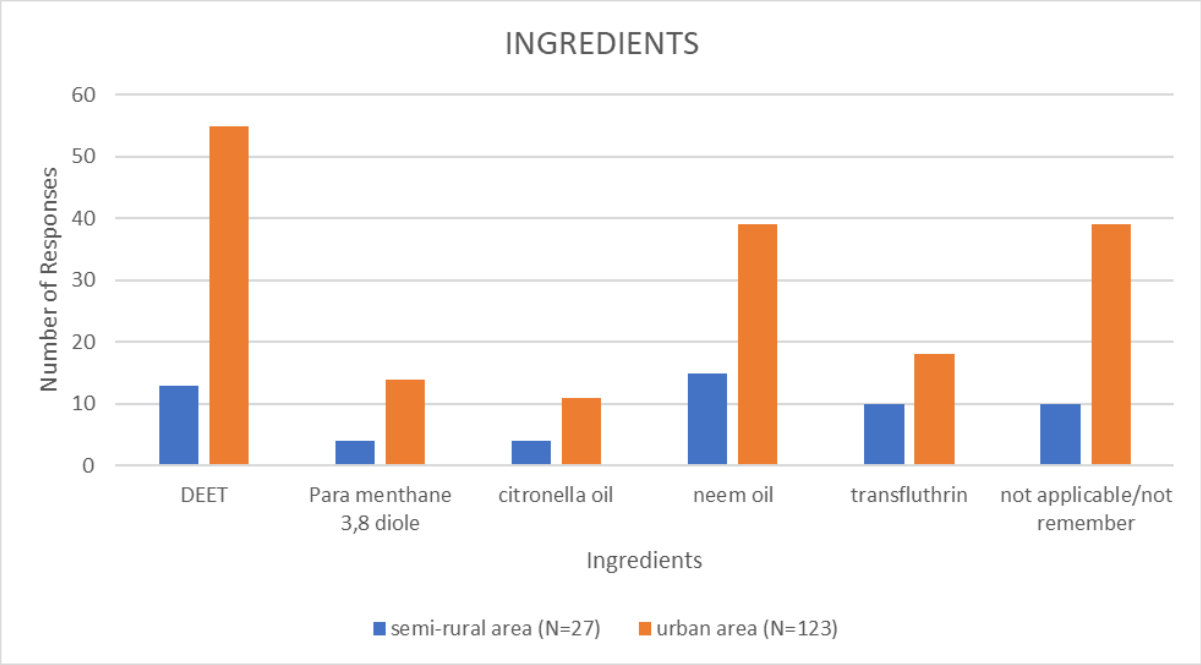

Figure 13 ***Types of Ingredients in Mosquito Repellent***

Table 13: statistical analysis of data of types of ingredients in mosquito repellents

Paired T-test	P-value (P<0.05)	Significantly effective
One tailed test	0.0185	Yes

From statistical analysis, observed P value was 0.0185 i.e., less than 0.05 so there is no significant difference available between types of ingredients used in mosquito repellents in urban and semi-rural areas. (Table 13)

Table 14: Awareness about the Precaution for the use of Mosquito Repellents

Have you observed precaution	**Semi-rural area (N=27)**	**Urban area (N=123)**
yes	18 (66.66%)	73 (59.34%)
no	9 (33.33%)	50 (40.65%)

According to the survey, 59.34% for urban and 66.66% for semi-rural area opted affirmation for observing the precautions that are labelled/mentioned on the packet. Meanwhile, the population of 40.65% for urban and 33.33% for semi-rural area do not observe the precaution. (Table 14, Figure 14)

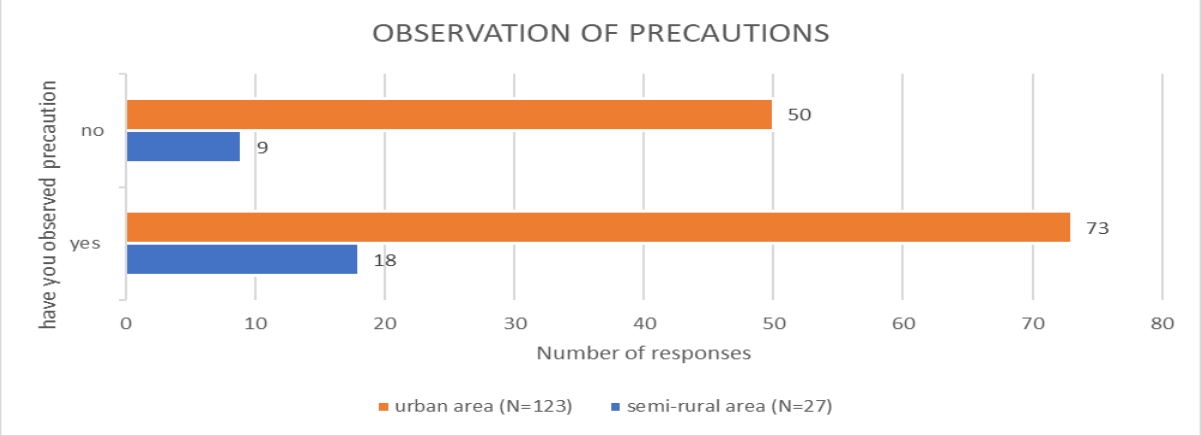

Figure 14 ***Awareness about the Precaution for the use of Mosquito Repellents***

Table 15: statistical analysis of data of awareness about the Precaution for the use of Mosquito Repellents

TYPE OF TEST	**T-STATISTICS (T)**	**P-VALUE (T<=t)**	**T-CRITICAL (t)**
One tail test	2.2857	0.1312	6.3137
Two tail tests	2.2857	0.2625	12.7062

This table of the t-test: Paired Two sample for Mean analysis shows that T-statistics (T) is less then T-critical (t) value, so our assumption illustrated that there is no difference in yes and no response of observing the precautions of mosquito repellent products in urban and rural areas in different forms. (Table 15)

Table 16: Side effects Observed by Participants

Have you observed side effects	**Semi-rural area (N=27)**	**Urban area (N=123)**
yes	16 (59.25%)	87 (70.73%)
no	11 (40.74%)	36 (29.26%)

According to the report, 70.73% for urban area and 59.25% for semi-rural area observed the predetermined severe adverse effects of various formulations; while, 29.26% and 40.74% respectively in urban and semi-rural area observed no side effects. (Table 16, Figure15)

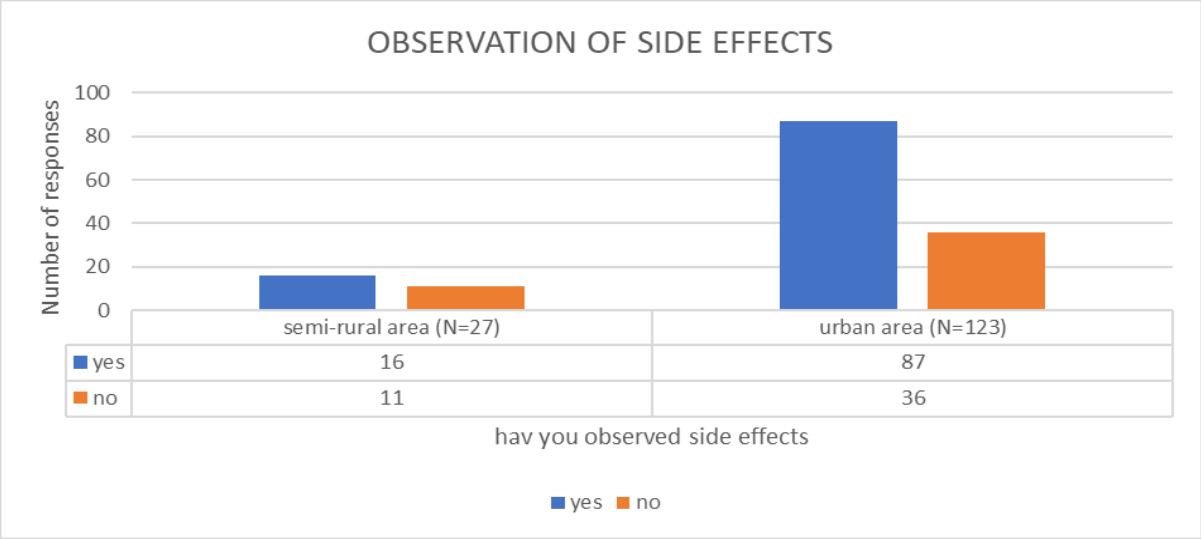

Figure 15 ***Side effects Observed by Participants***

Table 17: statistical analysis of data of Side effects Observed by Participants of mosquito repellents

TYPE OF TEST	T-STATISTICS (T)	P-VALUE (T<=t)	T-CRITICAL (t)
One tail test	1.2173	0.2188	6.3137
Two tail tests	1.2173	0.4377	12.7062

This table of the t-test: Paired Two sample for Mean analysis shows that Y-statistics (T) is less then T-critical (t) value, so our assumption illustrated that there is no difference in yes and no response of observing the adverse effects of mosquito repellent in urban and rural areas in different forms. (Table 17)

Table 18: Side effects Observed by Participants for Topical Formulations

In case of cream/ointment/gel	Semi-rural area yes (N=16)	Urban area yes (N=87)
skin irritation	12 (75%)	64 (73.56%)
allergies	6 (37.5%)	31 (35.63%)
rash	3 (18.75%)	27 (31.03%)
skin inflammation	5 (31.25%)	31 (35.63%)
not applicable	3 (18.75%)	15 (17.24%)

The given important data interprets that 73.56% in urban while 75% in semi-rural areas are facing skin irritation issue after constant use to topical formulation. Another side effect is Allergies which accounts for 35.63% in urban while 37.50% in semi-rural areas; rashes contribute the 31.03% and 18.75% respectively. Skin inflammation has been observed in 35.63% in unban and 31.25% in semi-rural areas. There are certain part of participants who did not noticed any adverse effect of used mosquito repellent, which associates 17.24% in urban while 18.75% in semi-rural areas. (Table 18, Figure 16)

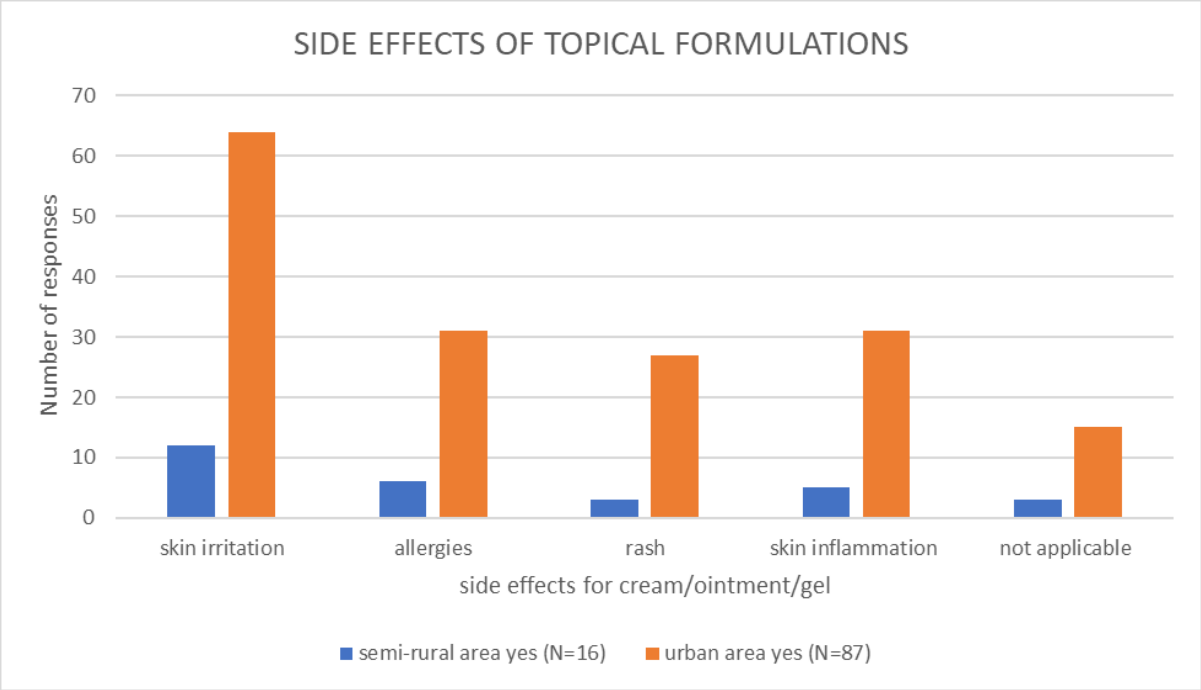

Figure 16 Side effects Observed by Participants for Topical Formulations

Table 19: statistical analysis of data of side effects observed by participants for topical mosquito repellent formulations

Paired T-test	P-value (P<0.05)	Significantly effective
One tailed test	0.0037	Yes

From statistical analysis, observed P value was 0.0037 i.e., less than 0.05 so there is no significant difference available between side effects observed in topical formulations of mosquito repellents used in urban and semi-rural areas. (Table 19)

Table 20: Side effects Observed by Participants for Coils

In case of coil	Semi-rural area yes (N=16)	Urban area yes (N=87)
cough	7 (43.75%)	46 (52.87%)
sore throat	6 (37.5%)	36 (41.37%)
wheezing	3 (18.75%)	23 (26.43%)
respiratory irritation	8 (50%)	24 (27.58%)
sneezing	4 (25%)	19 (21.83%)
suffocation	7 (43.75%)	38 (43.67%)
not applicable	1 (6.25%)	7 (8.04%)

According the statistics, 52.87% and 43.75% respectively in urban and semi-rural areas got the side effects of coughing; whereas, 41.37% and 37.5% people got the side effects of Sore throat. Wheezing accounts for 26.43% and 18.75% respectively; while 27.58% and 50% respectively for urban and semi-rural areas had been observed in Respiratory irritation. Sneezing and suffocation is observed by 21.83% and 43.67% respectively in urban, while 25% and 43.75% respectively in semi-rural areas. (Table 20, Figure 17)

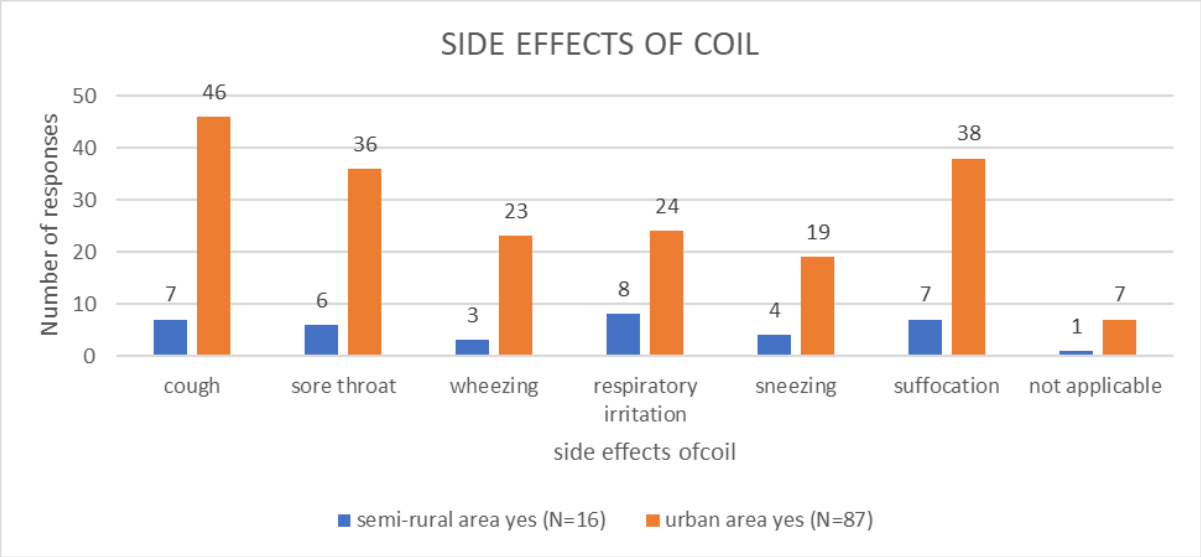

Figure 17 ***Side effects Observed by Participants for Coils***

Table 21: statistical analysis of data of side effects observed by participants for coils of mosquito repellent

Paired T-test	**P-value (P<0.05)**	**Significantly effective**
One tailed test	0.0215	Yes

From statistical analysis, observed P value was 0.0215 i.e., less than 0.05 so there is no significant difference available between side effects observed in coils of mosquito repellents used in urban and semi-rural areas. (Table 21)

Table 22: Side effects Observed by Participants for Aerosol

In case of Aerosol	Semi-rural area yes (N=16)	Urban area yes (N=87)
nasal irritation	8 (50%)	44 (50.57%)
coughing	9 (56.25%)	21 (24.13%)
eye irritation	11 (68.75%)	43 (49.42%)
not applicable	2 (12.5)	21 (24.13%)

According to data and graph, nasal irritation has been observed by 50.57% in urban and 50% in semi-rural areas; while coughing contributes 24.13% and 56.25% respectively. Lastly people also observed eye irritation in 49.42% in urban while 68.75% in semi-rural areas. (Table 22, Figure 18)

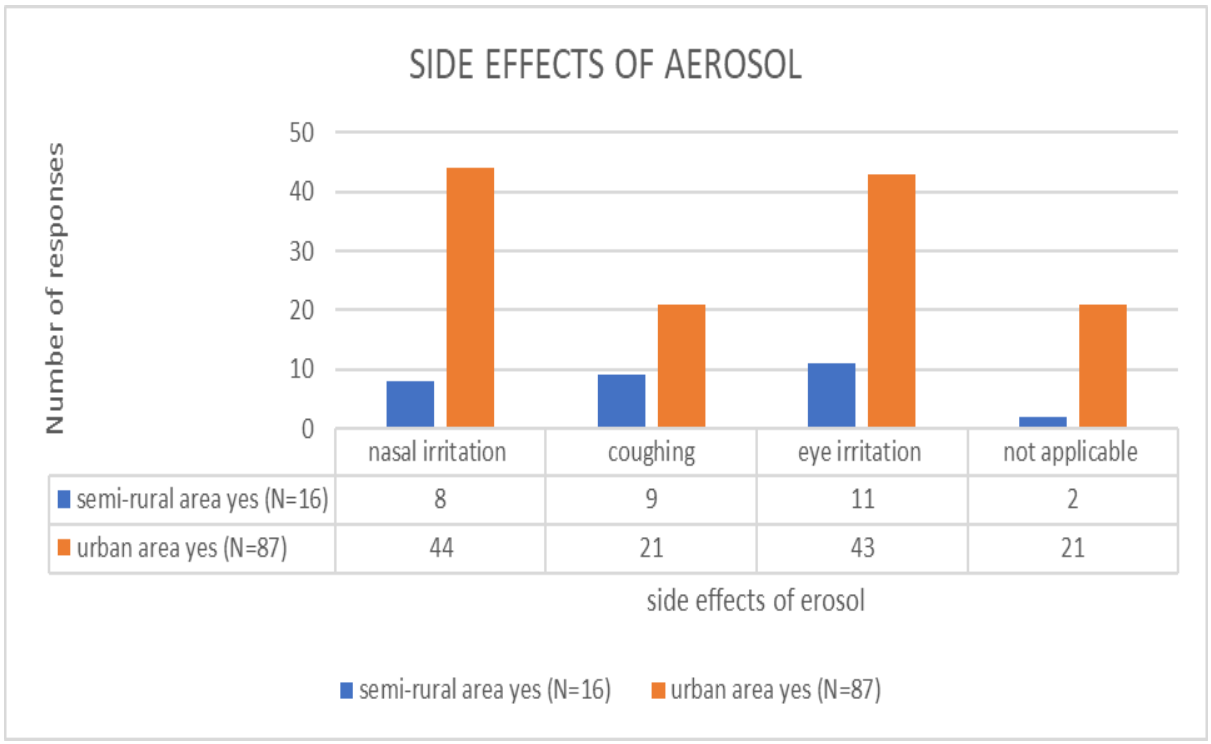

Figure 18 Side effects Observed by Participants for Aerosol

Table 23: statistical analysis of data of side effects observed by participants for aerosol mosquito repellent formulations.

Paired T-test	**P-value (P<0.05)**	**Significantly effective**
One tailed test	0.2070	No

From statistical analysis, observed P value was 0.2070 i.e., more than 0.05 so there is significant difference available between side effects observed in aerosol type formulation of mosquito repellents used in urban and semi-rural areas. (Table 23)

9. DISCUSSION

In current study, firstly we distributed our qualitative descriptive data into quantitative data by taking region as a variable. Regions have been bifurcated into two subtypes i.e., 1) Urban area (Ahmedabad, Gandhinagar, Ankleshwar, Surat, Vadodara, Mehsana, etc.) 2) Semi-Rural area (Junagadh, Jamnagar, Rajkot, Bhavnagar, etc.). Here we observed that, 88% participants are using mosquito repellent in one or other formulation. Most of the contributors are using that formulations once in a day, and least number of attendants using that formulation whole day. In accordance to the data, majority of participants are topical preparations (59.33%), followed by other formulations like electric coil (49.67%), aerosols (18.83%), normal coil (18.19%), fabric roll-ons (21.31%). Out of the responses, 52.66% associators are tends to be observed the content list of the marketed products, while remaining are not aware about the active particles. Moreover, the main constituent of the majority of marketed formulations comprises of DEET and NEEM oil. Furthermore, 60.66% volunteers had noticed the precautions that are mentioned behind the packet. Statistically, 68.66% of surveyors observed one or the other kind of side effects of different formulations. Mainly in terms of topical formulation like cream, gel, ointments, etc., skin irritations, allergies, and skin inflammation happen in promptly. While in case of coils, primarily coughing, sore throat, suffocation and respiratory irritations are heavily observed. When it comes to use of aerosol, nasal irritation and eye irritations are most recognized adverse effects.

In this study, the most appropriate formulation used among the population of both urban and semi-rural areas is topical preparations like cream, ointments, gels. Etc.; which is 61.78% and 48.14% respectively. These figures are compared with the studies done in Kerala where most used formulation of mosquito repellent is aerosol. The figure was nearly 75% to 85% in both urban and semi-rural areas. Even in remote areas, the current study found little use of smoke or conventional measures like plant that ward off insects. Because of how convenient contemporary chemical approaches are, they have likely been supplanted as a mosquito repellent. Other criteria of relevance include a deep understanding of and use of natural insect or mosquito repellent herbs. According to karunamoorthi et al., one of the greatest well-known ways among the residence of the area was the use of smoke, which involved the combustion of parts of plant such as leaves, stems, and roots. In our study, the most observed side effects are cough, sore throat, skin irritations, etc. Our revealed that nearly 68.66% persons who used

various repellents reported negative health impacts. The most frequent issue for topical formulation is skin irritation (73.78%) followed by allergies viz 35.92%. On the other side, in case of coils, cough has nearly 47% and suffocation at nearly 43%. Talking about aerosol formulations, nasal irritation tends to 50% side effect and eye irritation has 58.5%.

10. CONCLUSION

Users need to be closely monitored for any detrimental impacts of their personal protection gear. To create secure and reliable personal mosquito protection solutions additional investigation will also be required. Hence from the above the survey, observations, interpretation of the analyzed data and mechanism of action, cytotoxic effects of active ingredient called DEET which is used in almost 90% marketed formulation, here it is concluded that there must be a trials and experiments on p-manthane-3,8-diol (PMD). According to certain research undergone, it has been concluded that PMD is more beneficial rather than DEET. The other side of this survey justifies that majority of mosquito repellent applicants do not observe the written precautions which are mentioned at the backside of the pack. Moreover, many people do not know the active ingredient of the mosquito repellent formulation. Besides majority of people are not aware of benefits of herbal and natural mosquito repellents. There are certain limited products in market which comprises the mixture of both active ingredient DEET and herbal additives. But those have many limitations, hence there is a need for further investigation to upgrade the benefits and to minimize the adverse effects of DEET.

REFERENCES

1. Nakagawa, T., et al., *Insect sex-pheromone signals mediated by specific combinations of olfactory receptors.* 2005. **307**(5715): p. 1638-1642.
2. Sato, K., et al., *Insect olfactory receptors are heteromeric ligand-gated ion channels.* 2008. **452**(7190): p. 1002-1006.
3. Wicher, D., et al., *Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels.* 2008. **452**(7190): p. 1007-1011.
4. Bohbot, J.D. and J.C.J.F.i.c.n. Dickens, *Selectivity of odorant receptors in insects.* 2012. **6**: p. 29.
5. McIver, S.B.J.C.j.o.z., *Fine structure of pegs on the palps of female culicine mosquitoes.* 1972. **50**(5): p. 571-576.
6. Grant, A., et al., *Electrophysiological responses of receptor neurons in mosquito maxillary palp sensilla to carbon dioxide.* 1995. **177**: p. 389-396.
7. Grant, A.J. and R.J. O'Connell. *Electrophysiological responses from receptor neurons in mosquito maxillary palp sensilla*. in *Ciba Foundation Symposium 200-Olfaction in Mosquito-Host Interactions: Olfaction in Mosquito-Host Interactions: Ciba Foundation Symposium 200*. 2007. Wiley Online Library.
8. Takken, W. and D.J.J.o.t.A.M.C.A. Kline, *Carbon dioxide and 1-octen-3-ol as mosquito attractants.* 1989. **5**(3): p. 311-316.
9. Syed, Z. and W.S.J.C.s. Leal, *Maxillary palps are broad spectrum odorant detectors in Culex quinquefasciatus.* 2007. **32**(8): p. 727-738.
10. Bohbot, J., et al., *Molecular characterization of the Aedes aegypti odorant receptor gene family.* 2007. **16**(5): p. 525-537.
11. Bohbot, J.D. and J.C.J.P.O. Dickens, *Characterization of an enantioselective odorant receptor in the yellow fever mosquito Aedes aegypti.* 2009. **4**(9): p. e7032.
12. Jones, P.L., et al., *Functional agonism of insect odorant receptor ion channels.* 2011. **108**(21): p. 8821-8825.
13. Bohbot, J.D. and J.C.J.N. Dickens, *Odorant receptor modulation: ternary paradigm for mode of action of insect repellents.* 2012. **62**(5-6): p. 2086-2095.
14. Klun, J.A., A. Khrimian, and M.J.J.o.m.e. Debboun, *Repellent and deterrent effects of SS220, Picaridin, and Deet suppress human blood feeding by Aedes aegypti, Anopheles stephensi, and Phlebotomus papatasi.* 2006. **43**(1): p. 34-39.
15. Chauhan, K.R., et al., *Feeding deterrent effects of catnip oil components compared with two synthetic amides against Aedes aegypti.* 2005. **42**(4): p. 643-646.
16. Bissinger, B., et al., *Efficacy of the new repellent BioUD® against three species of ixodid ticks.* 2009. **48**: p. 239-250.
17. Khater, H.F., et al., *Commercial mosquito repellents and their safety concerns.* 2019. **1**: p. 1-27.
18. Khater, H.F.J.N.r.i.t.f.a.p., *Introductory chapter: Back to the future-solutions for parasitic problems as old as the pyramids.* 2017: p. 5-7.
19. Krajick, K., *Keeping the bugs at bay*. 2006, American Association for the Advancement of Science.
20. Islam, J., et al., *Mosquito repellents: An insight into the chronological perspectives and novel discoveries.* 2017. **167**: p. 216-230.
21. Maia, M.F. and S.J.J.M.j. Moore, *Plant-based insect repellents: a review of their efficacy, development and testing.* 2011. **10**(1): p. 1-15.

22. Davies, M., R. Soto, and R.J.J. Stewart, *Toxicity of diethyltoluamide-containing insect repellents.* 1988. **259**(15): p. 2239-2239.
23. Ellenhorn, M.J. and D.J.M.t. Barceloux, *Diagnosis and treatment of human poisoning.* 1997: p. 609-610.
24. Control, C.f.D., *Seizures temporally associated with use of DEET insect repellent--New York and Connecticut.* 1989. **38**(39): p. 678-680.
25. Gryboski, J., D. Weinstein, and N.K.J.N.E.J.o.M. Ordway, *Toxic encephalopathy apparently related to the use of an insect repellent.* 1961. **264**(6): p. 289-291.
26. Roland, E., J. Jan, and J.J.C.M.A.J. Rigg, *Toxic encephalopathy in a child after brief exposure to insect repellents.* 1985. **132**(2): p. 155.
27. Edwards, D. and C.J.C.p. Johnson, *Insect-repellent-induced toxic encephalopathy in a child.* 1987. **6**(6): p. 496-498.
28. Lietman, P.S., et al., *Reye-like syndrome associated with use of insect repellent in a presumed heterozygote for ornithine carbamoyl transferase deficiency.* 1980. **97**(3): p. 471-473.
29. Lipscomb, J.W., J.E. Kramer, and J.B.J.A.o.e.m. Leikin, *Seizure following brief exposure to the insect repellent N, N-diethyl-m-toluamide.* 1992. **21**(3): p. 315-317.
30. Koren, G., D. Matsui, and B.J.C. Bailey, *DEET-based insect repellents: safety implications for children and pregnant and lactating women.* 2003. **169**(3): p. 209-212.
31. Osimitz, T.G. and J.V.J.J.o.T.C.T. Murphy, *Neurological effects associated with use of the insect repellent N, N-diethyl-m-toluamide (DEET).* 1997. **35**(5): p. 435-441.
32. Briassoulis, G., et al., *Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children.* 2001. **20**(1): p. 8-14.
33. Bell, J.W., J.C. Veltri, and B.C.J.I.j.o.t. Page, *Human exposures to N, N-diethyl-m-toluamide insect repellents reported to the American Association of Poison Control Centers 1993–1997.* 2002. **21**(5): p. 341-352.
34. Schoenig, G.P., et al., *Teratologic evaluations of N, N-diethyl-m-toluamide (DEET) in rats and rabbits.* 1994. **23**(1): p. 63-69.
35. McGready, R., et al., *Safety of the insect repellent N, N-diethyl-M-toluamide (DEET) in pregnancy.* 2001. **65**(4).
36. Control, C.f.D. and Prevention, *Intrauterine West Nile virus infection--New York, 2002.* 2002. **51**(50): p. 1135-1136.
37. January, C.f.D.C.J.R., *West Nile Virus, pregnancy and breastfeeding.* 2006. **28**: p. 2006.
38. Fradin, M.S. and J.F.J.N.E.J.o.M. Day, *Comparative efficacy of insect repellents against mosquito bites.* 2002. **347**(1): p. 13-18.
39. Cheung, T. and B.J.C.P.J. Ruddock, *Use of insect repellents in children.* 2003. **136**(4): p. 14.
40. Onyett, H., et al., *Preventing mosquito and tick bites: A Canadian update.* 2014. **19**(6): p. 326-328.

APPENDIX

The following questions were asked in the survey that was conducted:

1)**Name**

2)**Age**

- 0-12
- 12-24
- 24-40
- 40-60
- 60+

3)**E-mail id**

4) **Educational Background from which you are belonging**

- 10th Pass
- 12th Pass
- UG Program
- PG Program
- PhD

5) **City from which you are belonging**

6) **Do you use Mosquito Repellants in any form?**

- Yes
- No

7) **If yes, how often do you use**

- Once a day
- Twice a day
- Whole day
- Seasonal

8) **Which type of formulation do you Currently use ?**

- Cream/Ointment/Gel
- electric coil(refill)
- Aerosol
- Normal Coil
- Fabric Roll on
- Other:

9) **Brand name of Mosquito Repellant you are currently using?**

- All Out by SC Johnson
- Goodnight by Godrej
- Mortein
- Odomos cream
- Maxo
- Other:

10) **Have you observed any Active Ingredient in the label of formulation packet**

- Yes
- No
- Maybe

11) **If Yes, then Which?**

- DEET
- Para menthane 3,8 Diole
- Citronella Oil
- Neem Oil
- Transfluthrin
- Not Applicable/Not Remember

12) **Have you ever gone through the precaution provided by company at back of formulation packet?**

- Yes
- No

13) **Do you observe any kind of Side effect after using Mosquito repellant of any Formulation?**

- Yes
- No

14) **In case of cream/ointment/gel do you observe any of the following side effects?**

- Skin Irritation
- Allergies
- Rash
- Skin Inflammation
- Not Applicable
- Other

15) **In case of Coil do you observed any of the following side effect?**

- Cough
- Sore Throat
- Wheezing
- Respiratory Irritation
- Sneezing
- Suffocation
- Not Applicable

16) **In case of Aerosol do you observed any of the following side effects?**

- Nasal Irritation
- Coughing
- Eye Irritation
- Not Applicable
- Other:

ABC

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