http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (16): 2714-2718, 2007
ISSN 1028-8880

Laboratory Determination of Protection Time in Four Chemical Repellents Against *Anopheles stephensi*

Mehdi Khoobdel and Nematollah Jonaidi
Military Health Research Center, Military Medicine Institute,
Baqiyatallah University of Medical Sciences, Tehran, Iran

Abstract: In the present study we determined the Protection Time (PT) and Failure Time (FT) of the DMP lotion, which is synthesized and formulated in Iran and it was compared with other products such as MIP60 and $Dimp_{31.7}$ lotions (commercial and current formulations of dimethyl phthalate) and trench pomade (a popular local repellent in Iran) against *Anopheles stephensi* Liston (main malaria vector in south of Iran) in laboratory condition. In this research which is an interventional and experimental study, the screen cage method was used to estimate PT and FT of repellents against *An. stephensi*. The following commercial formulations of chemical repellents were tested: Iranian DMP lotion (DMP60) (contains 60% dimethyl phthalate, 25% isopropyl alcohol, 5% twine 80 and 10% water), MIP60 and $Dimp_{31.7}$ lotions contains 60 and 31.7% active ingredient of dimethyl phthalate and trench pomade (a combination of N,N-diethyl-m-toluamide (DEET) and DMP). Test was done on human volunteers. In this test some defined amount of repellents applied on human volunteer's forearm and then was inserted in cage against mosquitoes biting to determine PT and FT. According to the results of this research, the PT of Iranian DMP60 lotion against *An. stephensi* was determined about 274 min (SE = ±8.04), which didn't have any significant difference with MIP60 and trench pomade, but it was significantly more than $Dimp_{31.7}$. Furthermore the FT of DMP60 against *An. stephensi* was determined about 327 min (SE = ±10.47), that in this case it had a significant deference with MIP60 lotion and trench pomade. The failure time of DMP60 was less than another two repellents. The Iranian DMP60 lotion can potentially compete with MIP60 and $Dimp_{31.7}$, but to increase the FT rate, its formulation need to be improved.

Key words: *Anopheles stephensi*, protection time, repellents, dimethyl phthalate, diethyl toluamide

INTRODUCTION

More than two billion people, especially in tropical countries are at risk from arthropod-borne diseases such as malaria, dengue hemorrhagic fever and filariasis (Service, 1993). The search for effective vaccines against these diseases is still in progress (Tawatsin *et al*., 2001). The principle approach to prevention of vector-borne disease is avoidance (Debboun *et al*., 2001). Personal protective measures, including repellents, are widely used to prevent the transmission of arthropod-borne diseases by minimizing the contact between human and vectors. The use of repellent is an obvious practical, convenient and economical means of preventing the transmission of these diseases to human (WHO, 1995).

During the past decades, N,N-diethyl-m-toluamide (DEET) has known as the most consuming and efficient repellent (Cockcroft *et al*., 1998). Researches have showed that using DEET for a long time can make tolerance in some insects, especially mosquitoes, like tolerance or resistance to insecticides. For instance, *An. albimanus* has been reported as being generally tolerant of many repellents, particularly DEET (Robert *et al*., 1991; Rutledge *et al*., 1983). But the laboratory research has proven that the majority of *Anopheles* are sensitive against DMP (Robert *et al*., 1991). Moreover some adverse effects induced by using DEET for a long time, or the risk of using it in combination with other chemical compounds such as pyridostigmine bromide are proven in experimental studies and can highly affect extensive use of DEET as an insect repellent (Santhanam *et al*., 2005; Abou-Donia *et al*., 1996). Using one repellent such as DEET continuously for a long time can cause tolerance in mosquitoes and adverse effects in human. Therefore, other chemical repellents such as DMP should be replaced as an alternative (Kalyanasundram *et al*., 1994; Curtis, 1992).

The aim of this study is determination of PT and FT in Iranian DMP lotion (DMP60) in comparison with other similar repellents such as MIP60 and $Dimp_{31.7}$ (foreign DMP lotion) and trench pomade (a popular local repellent and a combination of DMP and DEET) against *An. stephensi* (main malaria vector in south of Iran) in laboratory condition.

Corresponding Author: Mehdi Khoobdel, Molla Sadra Street, P.O. Box 19945/581, Vanak Sq, Tehran, Iran Fax: +98-21-88057023

MATERIALS AND METHODS

Mosquito: The mosquito which used in this study was laboratory reared female *An. stephensi* Liston (India strain). Adult's colonies were fed with 10% sucrose and maintained at 26-28°C, 70-80% humidity and with 12 h light: 12 h dark photoperiod. In order to test, the 7-8 days old nulliparous females, which starved 12-14 h before test, was used.

Test repellents: Four type repellents which were used in this study, prepared as:

Dimethyl phthalate is synthesized and formulated as a 60% lotion in a academic research center (Shahid Beheshti University of Medical Sciences) in Iran. This product contains 60% dimethyl phthalate (a I), 25% isopropyl alcohol, 5% Twin 80 and 10% water, which are all weight proportion. Two current commercial DMP lotion include $Dimp_{31.7}$ (Contain 31.7% DMP) obtained from Sterling Pharmaceuticals New Zealand and MIP60 (Shijiazhuang Unison Co depended to Teamax International Lt) purchased from drug stores. Also for comparative purpose, the active ingredient of DMP (99%) is obtained from Merck, Germany.

Trench pomade is a popular local repellent in Iran, which is bought from Tolid Daru Co, Iran. This repellent is a combination of 5-10% DMP and 20-25% DEET, approximately. This repellent has used in Iran over 20 years (Khoobdel *et al*., 2003, 2006). Its active ingredients attained from foreign companies and formulated in Iran.

Volunteers: The number of test subjects and their characteristics based on Environmental protection Agency was used (EPA, 2000). Four male volunteers with mean age of 26 years (with 24-32 years age ranges) were used to test. Essential educations about the tests were preformed and informed consent was given to them. Volunteers entered to study after scratch test (skin irritation test) to repellents by dermatologist.

Test procedures: This study, which is an interventional and experimental research, was conducted during 2005-2006 in laboratory condition in Iran. For determination of PT and FT, the screened cage method reviewed and revised it by Barnard (1999) and it was introduced by WHO as a standard method in 2000. In this study, the steps bellow is done sequentially to attain PT and FT:

- Four human volunteers were selected and prepared with essential educations.
- The cage of test (38×38×40 cm dimensions) was provided with used of wood frame cage with a sheet wood bottom, window screen (mesh size 256) on the top, back, left and right sides and a cotton stockinet or screen sleeve for access on the front.
- Two-hundred nulliparous 7-8 day old female which were unfed for 12 h, were placed in each cage by manual aspirator.
- One milliliter of commercial repellents DMP (1 g for trench pomade) was applied on the left forearms (an adult forearm is about 550-650 cm^2 depends on body size of them from elbow to wrist).
- After application, the repellent was allowed to dry for 5 min before proceeding. The treated forearm was inserted into the cage (a latex glove was used to protect the hand from mosquito bites) and the number of mosquitoes that land or probe the skin in 3 min was observed and recorded.
- The volunteer exited his forearm and rested for 30 min. The 3 min-test and 30 min-rest period was continued until happening 2 bites in one 3 min-test or one bite in one 3 min test, followed by another bite in an confirmatory test 30 min later, ends the test for that repellent.
- PT is calculated as that elapsed between the time of repellent application and the first confirmed mosquito bite or the time between repellent application and the observation period immediately preceding the first confirmed bite.
- A second cage of mosquitoes normally is used as a negative control to determine mosquito biting rate (biting pressure). The untreated forearm or lower leg of the subject was inserted into the cage and the number of mosquitoes that land and probe the skin in 30 sec was observed and recorded (insect bites must be prevented). The EPA recommended at least 10 land or probes within 30 sec for the subject to qualify as a test participant. Every hour this test should be repeated (EPA, 1999).
- After finishing PT (the 2nd bite), the test was continued until the 10th bite to determine FT.
- The interval between applying a repellent and the 10th bite is considered as repellent FT in accordance with its definition. The FT-PT index is also interpreted as the duration between the 1st bite (finishing PT) and the 10th bite. These two indexes are mostly used to compare similar repellents which have close specifications (such as PT).

In this study, every volunteer was tested once a day and by just one repellent. In each test fresh mosquitoes

were used. Because in some tests, using just one cage of mosquitoes, makes them tired, blocks their chemoreceptor and cause errors in biting behavior (Barnard and Dexue, 2004).

For each repellent, the tests were repeated 8 times with 4 volunteer and average was calculated. All volunteers were tested by 4 repellents included DMP60, MIP60, $Dimp_{31.7}$ and trench pomade. It is essential to mention that the interval between 4 repellents tests and repetition for each volunteer was at least 2-4 weeks. It allows volunteers not to face with large amount of repellents. Moreover, it gave the opportunity to rearing sufficient mosquitoes for test. After the tests, volunteers were advised to bath.

Statistical analysis: To compare FT/PT of repellents, analysis of variance (ANOVA) and Tukey test were used.

RESULTS

Protection Time (PT): In this study the PT of DMP60 against *An. stephensi* was determined 274 min (SE = ±8.04). Statistical analysis showed that PT of DMP60 doesn't have a significant difference with trench pomade and MIP60 ($p>0.05$), but It has a significant difference with $Dimp_{31.7}$ which has 31.7% of dimethyl Phthalate ($p<0.05$). The PT of $Dimp_{31.7}$ was about 209 min (SE = ±6.67), which is about 1 h less than DMP60 (Table 1).

Failure Time (FT): In this study, the FT of DMP60 against *An. stephensi* was determined about 327 min (SE = ±10.47). Based on statistical analysis, FT of DMP60 has a significant difference with trench pomade and MIP60 ($p<0.05$). Results showed that the FT of DMP60 finish earlier than other two repellents and is more than $Dimp_{31.7}$ with a significant difference. Also there was a significant different between FT of trench pomade and MIP60 ($p<0.05$). The FT of trench pomade (with different formulation from other repellents) against *An. stephensi* was determined about 466 min (SE = ±7.99), which is higher than all repellents Fts that used in this study such as MIP60 that was determined about 418 min (SE = ±11.15). Despite this repellent has 60% active ingredient of dimethyl phthalate (same as DMP60), but its FT is significantly more than DMP60 ($p<0.05$) (Table 2).

FT-PT index: The results showed that the FT-PT index of DMP60 against *An. stephensi* has a significant difference with MIP60 and trench pomade ($p<0.05$) and is less than

Table 1: Comparison between protection times of repellents against *An. stephensi*

Repellents	PT±SE (min)	Rang Min-Max
DMP60	274±8.04	227-320
MIP60	300±9.52	248-336
Trench pomade	277±8.67	225-327
$Dimp_{31.7}$	209±6.67	180-247

Table 2: Comparison between failure times of repellents against *An. stephensi*

Repellents	FT±SE (min)	Rang (min) Min-Max
DMP60	327±10.47	278-375
MIP60	418±11.15	389-491
Trench pomade	466±7.99	418-512
$Dimp_{31.7}$	258±6.55	230-288

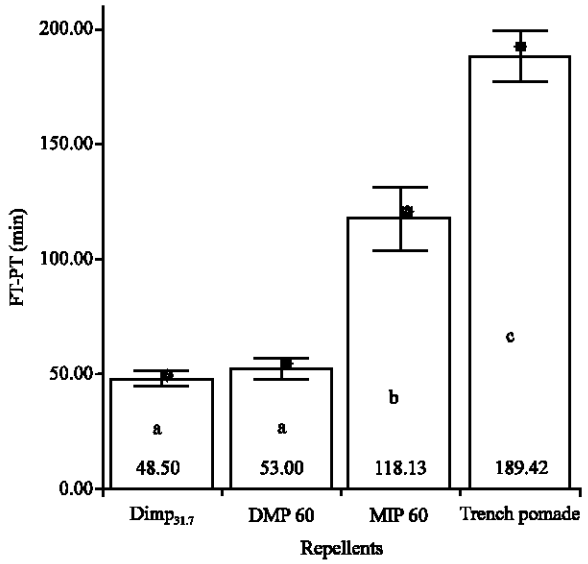

Fig. 1: Comparison FT-PT index of four repellents against *An. stephensi*, (a, b and c have a significant difference ($p<0.05$)) and Error bars show mean ±1.0 SE

them. It shows that for the DMP60 treated case after the first bite, the next bites (up to 10th bite) occur more quickly than other two repellents and its FT will finish in less than an hour after PT, but the FT of MIP60 and trench pomade continues up to 2-3 h, after finishing their PT.

FT-PT index for trench pomade is significantly more than all studied repellents ($p<0.05$) (Fig. 1). But there were no significant differences in FT-PT index between DMP60 and $Dimp_{31.7}$ (which contains 31.7% DMP active ingredient) ($p>0.05$) (Fig. 1).

No skin irritation, hot sensations or rashes were observed on the volunteers forearms treated with the DMP lotions and trench pomade by dermatologist during one year of the study period or in the six months, after time observations ceased.

DISCUSSION

This study showed that there is not a significant difference between DMP60 in comparison with MIP60 based on PT. Both repellents provide about 4-5 h protection against *An. stephensi*. There is not any significant difference in PT between DMP60 and trench pomade. It most probably depends on the trench pomade active ingredients.

Moreover, the results showed that the PT of DMP60 against *An. stephensi* is more than $Dimp_{31.7}$. DMP60 and $Dimp_{31.7}$ provided about 4.5 and 3.5 h protection, respectively. In others studies the FT of $Dimp_{31.7}$ is reported about 3-4 h (Spurr and McGregor, 2003).

$Dimp_{31.7}$ provides protection against *An. stephensi* about an hour less than DMP60, which most probably depends on their amount of the active ingredient, because active ingredient which is used in DMP60 and $Dimp_{31.7}$ is dimethyl phthalate, but the amount of it in $Dimp_{31.7}$ is approximately about half of DMP60.

According to this study, the FT of DMP60 against *An. stephensi* is less than MIP60, Although both lotions contains 60% dimethyl phthalate active ingredient and their PT do not have any significant difference, but their FT have a significant difference, so that FT of MIP60 against *An. stephensi* is 1.5 h more than DMP60. Due to the fact that amount of active ingredient in both lotions are equal, long duration of FT in MIP60 in comparison with DMP60 is most probably depends on some conservators and slow release substance that is added to repellent by producer companies.

The trench pomade FT is higher than all studied repellents which are 2.3 h more than DMP60 against *An. stephensi*. In addition to the type of active ingredient, it depends on the formulation too, because the formulation type is effective in repellent durability on skin (Coleman *et al*., 1994). Open formulations such as some repellents which can be solved in alcohols (usually pure ethanol) such as lotions or sprays, usually lose their FT earlier than other formulations (Douglas *et al*., 2005). In this study, DMP60 formulation was so, but in close formulations such as creams, polymer mixtures, microcapsules and microparticle formulation prolong the effect of repellents, because their active ingredient release slowly (Gunther, 2003).

FT of trench pomade, which is combined with DMP and DEET, is longer than other repellents that used in this study. Probably PT and FT for combination of DEET and DMP such as trench pomade is more than DMP alone. Our finding in this section of study is only limited to one species (*An. stephensi*) with 4 chemical repellents and only one of them was a combination of DMP and DEET. It needs to have further investigation.

The FT-PT index of Iranian DMP lotion (DMP60) against *An. stephensi* was significantly less than similar foreign DMP lotion. It point out that Iranian DMP lotion formulation need to be improved.

Finally, present study showed that Iranian DMP lotion (DMP60) which is prepared in Iran has essential potential to compete with MIP60 and $Dimp_{31.7}$, but its FT is less than MIP60, which can be probably improved by adding some additional materials or changing their formulation.

ACKNOWLEDGMENTS

We would like to thank Military Health Research Center-Baqiyatallah University of Medical Sciences for their financial supports, Mr. Babak Sharif for his cooperation in edit and translation of this manuscript and the volunteers Misters: Hamed Ramezani, Hossein Kashefi, Allahyar and Mohammad Abtahi.

REFERENCES

Abou-Donia, M.B., K.R. Wilmarth, A.A. Abdel-Rahman, K.F. Ensen, F.W. Oehme and T.L. Kurt, 1996. Increased neurotoxicity following concurrent exposure to pyridostigmine bromide, DEET and chlorpyrifos. Fundamental Applied Toxicol., 34: 202-222.

Barnard, D.R., 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). J. Med. Entomol., 36: 625-629.

Barnard, D.R. and R. Dexue, 2004. Laboratory evaluation of mosquito repellents against *Aedes albopictus*, *Culex nigripallpus* and *Ochlerotatus triseriatus*. J. Med. Entomol., 41: 726-730.

Cockcroft, A., J.B. Cosgrove and R.J. Wood, 1998. Comparative repellency of commercial formulations of, permethrin and citronella against the mosquito *Aedes aegypti*, using a collagen membrane technique compared with human arm tests. Med. Vet. Entomol., 20: 289-294.

Coleman, R.E., A.L. Richards, G.J. Mangnon, C.R. Maxwell, M. Debboun, T.A. Klein and R.A. Writz, 1994. Laboratory and field trials of four repellents with *Culex pipiens*. J. Med. Entomol., 31: 17-22.

Curtis, C.F., 1992. Personal protection methods against vectors of disease. Med. Vet. Entomol., 80: 543-553.

Debboun, M., R.E. Coleman, P.K. Gupta and D. Strickman, 2001. Soldier acceptability of camouflage face paint combined with DEET insect repellent. Mil. Med., 166: 777-782.

Douglas, H.D., J.E. Co, T.H. Jones, W.E. Conner and J.F. Day, 2005. Chemical odorant of Colonid Seabid repels mosquitoes. J. Med. Entomol., 42: 647-651.

Environmental Protection Agency (EPA), 1999. Product performance test guidelines: OPPTS810. 3700, Insect repellents for human skin and outdoors premises, No. 712-C: 99-369. http://www.epa.gov/epahome/research.

Environmental Protection Agency (EPA), 2000. Insect repellent product performance testing guideline evaluation. FIFRA scientific Advisory Panel Meeting, SAP report, No.00-02B: 44-56.

Gunther, N., 2003. Use of repellents as prophylactic agents. Parasitol. Res., 90: 40-48.

Kalyanasundram, M., R. Srinivasan, S. Subramanian and K.N. Panicker, 1994. Relative potency of DEPA as a repellent against the sandfly *Phlebotomus papatasi*. Med. Vet. Entomol., 8: 68-70.

Khoobdel, M., H. Fajrak, H. Ladoni, M. Shayegi and R. Asadzadeh, 2003. A new method for military personal protection against insects. Iran Mil. Med., 5: 147-155.

Khoobdel, M., M. Shayeghi, H. Vatandoost, Y. Rassi, M.R. Abaei, H. Ladonni, A. Mehrabi Tavana, S.H. Bahrami, M.E. Najafi, S.H. Mosakazemi, K. Khamisabadi, M.R. Akhoond and S. Azari Hamidian, 2006. Field evaluation of permethrin-treated military uniforms against *Anopheles stephensi* and 4 species of *Culex* (Diptera: Culicidae) in Iran. J. Entomol., 3: 108-118.

Robert, L.L., J.A. Hallam and D.C. Seeley, 1991. Comparative sensitivity of four *Anopheles* to five repellents. J. Med. Entomol., 28: 417-420.

Rutledge, L.C., D.M. Collister, V.E. Meixsell and H.G. Eisenberg, 1983. Comparative sensitivity of representative mosquitoes to repellent. J. Med. Entomol., 20: 506-510.

Santhanam, A., M.A. Miller and G.B. Kasting, 2005. Absorption and evaporation of N, N-diethyl-m-toluamide from human skin *in vitro*. Toxicol. Pharmacol., 204: 81-90.

Service, M.W., 1993. Mosquitoes (Culicidae). In: Insects and Arachnids. Lane, R.P. and R.W. Crosskey (Eds.), Medical Chapman and Hall, Londone, pp: 723.

Spurr, E.D. and P.G. McGregor, 2003. Potential invertebrate antifeedants for toxin baits used for vertebrate pest control: A Literature review. Science for Conservation. New Zealand, 232: 1-36.

Tawatsin, A., S.D. Wratten, R.R. Scott, V. Thavara and T. Techadamrongsin, 2001. Repellency of volatile oils from plants against three mosquito vectors. J. Vector Ecol., 26: 76-82.

WHO., 1995. International travel and health vaccination requirement and health advice. World Health Organization, Geneva.