RESEARCH ARTICLE

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Comparing Larvicidal Effect of Methanolic Extract of the Different Parts of Henbane (*Hyoscyamusniger L.*) Plant on *Anopheles* spp Larvae in Vitro

Mahmoodreza Behravan[a], Amin Baharshahi[b], Mohammadreza Vaezi-Kakhki[b], Ali Naghizadeh[c,d*]

[a]Birjand Infectious Diseases Research Center & Department of Microbiology, Birjand University of Medical Science, Birjand, Iran.
[b]Department of Biology, Faculty of Basic Science, Hakim Sabzevai University, Sabzevar, Iran.
[c]Medical Toxicology and Drug Abuse Research Center, Birjand University of Medical Sciences, Birjand, Iran.
[d]Department of Environmental Health Engineering, School of Health, Birjand University of Medical Science, Birjand, Iran.

***Correspondence** should be addressed to Dr. Ali Naghizadeh, **Email:** al.naghizadeh@yahoo.com;

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Background & Aims of the Study: Malaria is an infectious disease by fever, chills and anemia; and splenomegaly genus Plasmodium parasite is the agent of it. One of the easiest and least expensive methods for preventionof this disease is removing the vector that usually has been done by insecticides and chemical pesticides, but now days due to the harmful effects of toxic chemicals, it is currently trying to use organic toxic and plant compounds in order to combat the pests. So, in this study *Hyoscyamus niger* was used in order to destroy the larvae of this insect and positive results were compared these plants together.

Materials and Methods: *Hyoscyamus niger* was collected and dried to extract by methanol in a rotary evaporator. Mosquito larvae were collected from stagnant water pits and ponds around the Birjand city, South Khorasan of Iran in order to apply the relevant tests identity and isolated Anopheles spp mosquito larvae.

Results: *Hyoscyamus niger* positive effect was destroying on the *Anopheles* spp larvae and between obtained results, the most powerful extract for destroying the mosquitoes *Anopheles* spp larvae was the flower extract of henbane (LC_{50}=0.07) and the weakest extract was the extract of the root of henbane (LC_{50}=0.78).

Conclusion: According to the results it is recommended the flower extract of henbane as a toxic, organic and natural compound to fight the larvae of Anopheles spp mosquito larvae which used in the other parts of these plants more stronger and more effective.

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Background

Clinically, malaria is a hemolytic feverish disease associated with fever, chills, anemia and spelenomegaly, and its agent parasite is named *Plasmodium* spp which lives inside the red blood cell and it is transmitted by blood-eating mosquitoes belonging to *Anopheles* genus (1). Given that nearly one billion people of the world's population at risk of malaria and about two million people die as a result of this disease. Malaria can be considered as the most important parasitic disease in the world (2). In Iran, 19 species of *Anopheles* have been reported that 7 species of them have been recognized as malaria vectors, and *Anopheles stephensi* has been reported as the most important vectors in Iran (3). The plant *Hyoscyamus niger* that is also known as henban is one of the most important medicinal plants of the family of Solanaceae, which it is spread

widely in Asia and Europe. The importance of this plant is due to high special alkaloid compounds such as hyoscyamine and scopolamine that they are used in making the drugs of hyoscyamine sulfate and scopolamine Hydrobromide that are anti-cholinergic and anti-spasmodic drugs, respectively. This plant has anti-spasmodic, analgesic and sedative properties (4). Anglo-Saxons named this plant as Henbane, since hens were paralyzed after eating the seeds of this plant (5). One of the ways to prevent the outbreak of malaria is fight against its vector mosquito. For this purpose, toxins and chemical insecticides are always used. However, they have devastating and harmful effects on the environment and the ecosystem, and much use of these toxins cause the carrier resistance against it (6). Therefore, the aim of this study is the use of different parts of henban plant as a larvicidal to fight with *Anopheles*spp larvae. After conducting the relevant test, results were recorded and finally larvicidal effects of different parts of these two plants were compared.

Materials & Methods

Extraction

Different parts of the plant henbane were collected in May 2015 of the foothills of the Tabas village with geographic coordinates of 36° 24 '15.9 "N, 57° 42' 30.5 'E on the outskirts of Sabzevar city of Iran. After washing with water for 15 days, they were kept at room temperature and away from sunlight to be dried completely. One sample of the plant was sent to botanical herbarium of Ferdowsi University of Mashhad to identify and to determine the species. The species code was 32493 for henbane. Finally, the extraction was started from this plant according to modified species (7). Different parts of the plant including root, stem, leaves, and flowers were separated after drying and they were grounded separately.

Then, 100 g of powder for every part of the plant was mixed with 500 ml of methanol and it was kept away from sunlight for 72 hours, and it was kept at temperature of 20-25 ° c. Then, the obtained extract was passed through Whatman filter paper No.2. Finally, Rotary device was used for drying and removing the solvent, and tar-like and thick extract was obtained finally.

Hunting Anopheles larvae

To prepare the *Anopheles* mosquito larvae in August 2015, the researcher referred to fresh and stagnant water ponds and puddles around the Birjand city with geographical position of 32° 49'42.8 "N, 59° 10'38.6" E and ladling method was used to hunt and collect larvae of mosquitoes. Then, larvae were transferred to parasitology & entomology laboratory of Birjand University of Medical Sciences and the third and fourth instar larvae of *Anopheles* were identified and separated according to the World Health Organization (WHO) medical entomology expert (8).

Measurement of larvicidal

The concentrations of 0.25, 0.5, 1, 2, and 4 mg/ml were prepared from extracts of root, stem, leaf and flower of henbane by distilled water according to the recommendation of the WHO (9). In each plate, one concentration of extract related to each part of the plant was poured and 10 Anopheles larvae were placed inside of each plate and 10 larvae as control were placed in a plate containing distilled water. Then, plates were transferred to refrigerator and temperature of +4°c away from the light. Then, at 6, 12, 24, 48 and 72 hours, being alive or dead of larvae was ensured by hitting the tip of the needle to them and watching their reaction to stimuli. Then, the obtained results were recorded (10) and the experiment was repeated three times; then, to any part and any concentration, mean number of killed larvae was taken and it was analyzed, using Excel 2010.

Results

This study is a clinical trial study conducted on larvae of *Anopheles*spp in the Birjand city in

laboratory conditions. Our findings showed that the root of methanolic extract of henbane root with increasing concentration in the first 6 hours had no significant impact compared to the regression line on larvae of *Anopheles* and P value was greater than 0.05. However, P_{value} was equal to 0.02 at 12 hours, indicating the significance of the results and LC_{50} = 5.06ppm, and it became p=0.07 at 24 hours. It suggests that our results have become far from significance and finally p was equal to 0.01 indicating again significance of the results and its LC_{50} was obtained 1.03 and 0.87 ppm respectively for 48 and 72 hours. Methanolic extract of henbane with increasing concentration in the first 24 hours had no significant effect on Anopheles larvae and after 48 hours, p value was 0.08 that was not significant still, but it was 0.01 after 72 hours indicating the significance of our results and its LC_{50} was obtained 0.74. Considering the stem extract, methanolic extract of henbane with increasing concentration in the first 24 was p=0.06, that was very close to significance, but it was not significant and concentration was obtained p=0.31 after 48 hours that was so far from significance and it was p=0.02 after 72 hours that indicated the significance of the results and its LC_{50} was obtained 0.9. The results obtained for the methanol extract of henban in the first 24 hours of increased concentration had no impact on *Anopheles* larvae (P_{value}>0.05) and after 48 hours, P_{value}=0.02, which indicates the significance of our results to increased concentration and its LC50 was 0.26. However, after 72 hours, P_{value}=0.06, indicating that the result was far from significance (The above results are given in Table 1). Our findings showed that in addition to increased concentration, increased exposure time of extracts caused more larvae to die, and in this regard, the methanol extract of henbane flower at all times (6, 12, 24, 48 and 72 h) had more power in destroying the larvae of *Anopheles* compared to methanol extract of other parts of henbane (Figure 1).

Table 1) Compare LC_{50} extract of extract of the different parts of henbane (*Hyoscyamus niger* L.) plant in third and fourth instars larvae of *Anopheles*spp according ppm, at 6 to 72 hours with confidence P <0.05

Extract	LC_{50} / P_{value}	Time exposure extract				
		6h	12h	24h	48h	72h
Root	LC_{50}	114.11	5.06	2.75	1.03	0.78
	P_{value}	0.18	0.02	0.07	0.01	0.01
Leaf	LC_{50}	-	43.35	10.84	2.47	0.74
	P_{value}	-	0.42	0.28	0.04	0.04
Stem	LC_{50}	0.11	0.11	0.11	17.79	0.09
	P_{value}	0.6	0.6	0.6	0.31	0.02
Flower	LC_{50}	2.57	1.74	0.54	0.26	0.07
	P_{value}	0.18	0.12	0.13	0.02	0.06

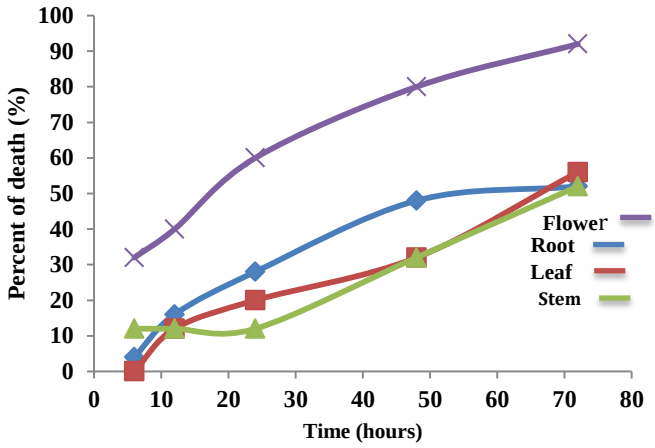

Figure 1) The effects of methanol extracts of different parts of henbane (*Hyoscyamus niger* L.) in the removal of the third and fourth instar larvae of *Anopheles*spp toward increasing concentrations and increased time exposure to the extracts.

Discussion

Vectors are important factors in transfer of different pathogens in Iran, and the issue has become a major health challenge in our country (11,12). One of the most important ways to prevent parasitic diseases such as malaria is fight against vectors that it is *Anopheles* in the case of malaria (13). Chemical toxins always have been used to fight against these vectors, but the effect of these compounds on the environment and the ecosystem is adverse and harmful much use of these toxins cause the vector resistance against it (10). Therefore, in recent years, scientists are looking to find a safe alternative for the environment and human health and other non-target organisms that the most important of these alternatives is the use of different methods of biological control and the use of organic toxins obtained from plants. These organic toxins, often at low concentrations, kill the arthropods, while they have no danger for bigger animals. In addition, these extracts obtained from plants such as chemical toxins often have no accumulation properties and cause no danger to the ecosystem and consumer beasts in the long term. In this study, it was generally determined that the methanolic extract of the henbane flower compared to other parts of the plant has a greater impact on the elimination of larvae of *Anopheles* spp; its LC_{50} was obtained 0.07 ppm at 72 hours. In addition, the weakest extract was the leaf extract of the plant with LC_{50}=0.74. Although, many reports about anti-larval activities on aromatic plants properties are increasing in the world but no report has been presented about the larvicidal effect of the henban. Considering the larvicidal effect on aromatic plants in Iran and the world, many studies have been conducted. For example, in a study conducted by 6. Fakoorziba et al in 2015 at Shiraz University of Medical Sciences, larvicidal effect of five different extracts of oleander plant prepared by five solvents acetone, petroleum ether, benzene, chloroform and water was examined against the larvae of *Anopheles stephensi*. Results showed that the benzene extract of flowers of this plant had the largest power and chloroform extract of leaves of this plant had the lowest larvicidal effect (6). These results are in line with the results of our study, so that in our study, the flower extract effect of the plant was more than larvicidal effect of leaf extract. In addition, in a study conducted by Bagari et al in 2013 in the country of Morocco, the toxic effects of oleander leaves on grasshopper were examined on the locust larvae and results showed that oleander leaf has positive impact in controlling the grasshopper (14). In a study conducted in 2010 by Dulger et al, methanolic extract of henbane seeds were used to inhibit the activity of 6 species of fungal pathogens, *Candida* spp and it was concluded that the seed extract of this plant has strong antifungal activity against every 6 species (5). This result was in line with the results of our study that indicated the toxicity of different parts of the plant. In another study conducted in 2010 by Lokesh et al in India, larvicidal activity of oleander and fenugreek leaves (*Trigonella foenum*) against mosquito larvae was studied and they concluded that leaves of both plants had high larvicidal effect and combination of these two

extracts had greater larvicidal (15). In some studies to test extracts on mosquito larva, it was attempted to reproduce a species of mosquitoes and reproduced larvae of the same species which were used for testing the impact of different extracts on mosquito larva (16) that this indicates the impact of extract on the same species. However, as the aim of our study was to eliminate the larva of a species of *Anopheles* spp and to introduce an organic and natural compound to fight against larvae of all *Anopheles* spp in the Birjand city, and as *Anopheles* mosquito fauna have not been identified in this city and the dominant pathogenic species is not specified in this area, the larvae mosquitoes were obtained from the fresh and stagnant water of pit and ponds in the Birjand city. Then, the third and fourth instar larvae related to the genus *Anopheles*spp were identified by entomology expert, and the impact of the various concentrations of different parts of this plant extract on the *Anopheles* larvae was examined with 3 replications. The results were recorded and the mean mortality for each concentration was the base of probit regression analysis.

Conclusion

Finally, considering that this plant is a native of Iran, it can be used as a native tool to fight against *Anopheles*spp larvae and advantage of this insecticide over its chemical types is its biodegradability. It seems that due to having alkaloid compounds such as hyoscyamine and Scopolamine, this plant can be used to eliminate *Anopheles*spp larvae. These findings can be used as an effective solution to fight against *Anopheles*spp larvae as vectors of malaria disease in developing countries without imposing environmental damage.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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