



The membrane transport of lemon grass palladium metallic nanoparticles and their synergistic action on plasma membrane integrity concerning endosmosis and fatality in *Culex pipiens* mosquito larvae

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Abstract

The lemon grass palladium metallic nanoparticles (NPs) are 20 ± 1 nm in size, oval shape, and can enter a cell via a plasma membrane. The essential lipid bilayer is primarily composed of proteins and lipids. The protein portion of the membrane facilitates the transport of any molecules from the outside to the inside of the cell. The transport form of proteins is called transmembrane proteins, ion channels that allow molecules inside the cell. The nanoparticle also follows the same route to enter the cell and pass through protein structures. Once the NPs enter the cell, they initiate their actual mechanism for the degradation of the plasma membrane in mosquito larvae by producing reactive oxygen species (ROS) via a sequence of reactions, and finally, due to endosmosis and loss of equilibrium fluid from the outside environment enter the cells, of mosquito larvae, and it kills the mosquito larvae. In the current research, we have explored the exact mechanism of ROS and the consequent mechanism for the death of larvae. Finally, we concluded that endosmosis is the fatal entity in larvae.

Keywords: membrane transporters, LgPd NPs, aquaporins, Ion channels, ROS, Endosmosis

Introduction

Today's world is of nanotechnology. Most things in medicine, information technology and electronics are nano in their product size for the comfortable and effective utilization of their products and services. Nanotechnology is not only helpful in empowering the world in effective management of the benefits but also in delivering better results from previous scenarios of events [1]. Nanotechnology primarily reduces its size for the better achievement of results. Therefore, the NPs came into innovation, mainly if we talk about physiology and medicine; the drugs improve their life span and half-life and effectively deliver the medications to goal-oriented targeted organs [2]. The NPs range from 10 to 100 nm in size with a polydispersity index (PI), preferably negative. Such NPs passively diffuse in living cell membrane and start their action simultaneously [3]. The NPs are designed in such a way that they will only hit the target region and will not harm any other unspecified areas. Hence, nanotechnology is improving their treatment modalities, enhancing their theranostic approach. The living cells of animals are composed of a lipid bilayer as an external covering; in the case of plants and bacteria, this becomes a secondary structure. The cell wall is the primary structure in plants and bacteria as an outer membrane. The plasma membrane comprises different components like lipids, cholesterol, proteins and carbohydrates. The lipids surrounding the envelope of the cell form a lipid bilayer, and cholesterol is merged between the membrane to facilitate membrane fluidity. The proteins are of various types, either joined between this bi-layer or present on either side of the membranes. These proteins are referred to as extrinsic and intrinsic proteins. A few more proteins are also a part of these structures, called lipid-anchored proteins [4]. In some proteins, the carbohydrates attached to the periphery are called oligo proteins. The proteins also form ion channels and transporters in the lipid bilayer to enter the water, glucose, nutrients etc., across the membrane; for example, porin proteins (aquaporins) form water channels and GLUT transporters for the transport of glucose [5]. The NPs also move across the membrane via passive diffusion, ion channels, transporters, etc. The plasma membrane is semi-permeable and selectively permeable for the exterior medium. The particular NPs' size enables them to enter the cell via its cell envelope and perform a specific function [6]. In the case of cancer theranostics, the NPs targeted the newly forming cell structure via the enhanced permeation and retention (EPR) effect. These NPs then cause mortality in such cells, degrade their tissue empire, and aid in improving the standard conditions. Due to their leaky vasculature, the phenomenon is that the newly developing arteries, blood vessels and lymphatics allow NPs to integrate into the cells. Therefore, these NPs will show their action and kill the cancer cells [7]. Depending on the aims and objectives, the NPs are designed. They will enter the cell membrane via various routes such as oral contact, muscular and peritoneal injections, heat shock treatment etc. The NPs travel through the membranes and enter the cell's internal environment. The green synthesized lemon grass palladium metallic NPs (LgPd NPs) are biocompatible with the living cells of higher animals but deadly for the parasitic organisms already proven by our previous experiences and published articles [8]. It was observed

that the LgPd NPs were biocompatible with the zebrafish and their embryos, and a harmful and massive killing effect was observed in *Culex* and *Aedes* mosquito larvae. The biocompatibility depends on the organelles present; the animal facilitates the exact action of these particles on the damaged portion of the animals, and pathetic diseased conditions, will be cured with these NPs. Therefore these NPs show a biocompatible nature is proposed animals. After the completion of their action, they will be removed from the body by the action of the kidney and liver is called renal clearance and hepato-biliary clearance, respectively. In small organisms, for instance, mosquitoes, these NPs perform a deadly function. They enter the larvae body due to the EPR effect and accumulate in the body structure; later, the NPs damage the cell's plasma membrane and degrade it [9]. Finally, due to the endosmosis mechanism, the entire animal fails to survive and dies. The ROS well played a role here in damaging the membrane. LgPd NPs enable the production of ROS, and ROS will degrade the membrane, and due to the osmotic difference, water will enter the body of the cell and swelling results in the bursting of the cell, and the death of an animal occurs. Endosmosis is the process by which liquid moves along the concentration gradient. Therefore, for instance, if freshwater fish, for example, zebrafish if kept in marine water, then the fluid from the outside environment will start to move inside the body cavity of the animal; consequently, the endosmosis occurs, and finally, the body swells, and the animal dies soon due to hypertonic conditions [10]. Therefore it was recommended that equilibrium must be maintained with the outer environment so that it results in the betterment of the survival of the animal. The LgPd NPs will not directly cause the endosmosis, but it stimulates the production and then the produced ROS promote leaky structure in the lipid bilayer. Further, through such leaky intervention, the liquid moves inside the cell area, and due to a decrease in surface area to volume ratio, the fluid enters the cell volume and will finally burst into pieces. This phenomenon was already studied in our previously published data for killing mosquito larvae [11].

Materials and Methods

Light microscope, *Culex pipiens* mosquito larvae, LgPd NPs, Beakers, Petri dishes etc.

Identification of mosquito larvae

Mosquito larvae belong to the *Culex* genus and *pipiens* species observed and identified according to the previously published data.

Culturing of mosquito larvae

The *Culex pipiens* mosquito larvae were cultured with the help of previously published data. In brief, the aquarium wastewater was collected in a jar and kept overnight in a dark place. Where *Culex* mosquitoes breed, and in the morning, the egg rafts were collected, and these rafts were kept for culture.

Synthesis and characterization of LgPd NPs

The LgPd NPs were synthesized biogenically and characterized with the help of TEM.

Transport of LgPd NPs through the plasma membrane

The synthesized NPs were transferred into the mosquito larvae plasma membrane. The plasma membrane consists of ion channels, aquaporins and transporters that transport medium, water, solutes, nutrients, etc. The green LgPd NPs enter the cell membrane via such transporters and accumulate in the cell. The EPR effect of developing larvae helps in the accumulation of these NPs in the larval structures and facilitates the production of ROS.

Production of ROS in mosquito larvae

The mosquito larvae were subjected to the action of LgPd NPs, and therefore, the NPs initiate ROS production in the larvae's internal environment. The temperature of the surrounding environment of mosquito larvae was raised to 37 °C with the help of an oven, which promotes the action of NPs and initiates ROS production. Therefore, in each petri dish, 10 mosquito larvae were kept, and 10 µg/mL of LgPd NPs solution was added to each dish. A similar experiment was repeated thrice and measured the death profile of larvae.

Mechanism of endosmosis and lysis of mosquito larvae

The action of LgPd NPs results in larvae lysis, which can be observed in the light microscope. The NPs were added to each petri dish with 10 numbers of *Culex pipiens* larvae and waited for the subsequent 48 hr incubation. After and between incubation, larvae were observed for the NP's effect on their structures. The images were captured and stored for further record.

Results and Discussions

Synthesis and characterization of LgPd NPs

The LgPd NPs were synthesized and characterized according to the previously published data. The size of the synthesized NPs was 20 ± 1 nm in size. These NPs were characterized with the help of TEM, and the results were analysed, and it was predicted that the synthesized NPs were oval [8].

Transport of LgPd NPs through the plasma membrane

The LgPd NPs, due to nano size, can be entered through the plasma membrane. The extrinsic and intrinsic proteins provide the way for the travel of these NPs. The NPs safely move inside the cell of larvae and accumulate inside. The transport of these particles was passive due to their size. The accumulation of NPs inside the cell was due to the EPR effect already proposed in our previous results and published data. There was no need for an osmosis gradient for the entry and exit of LgPd NPs; the EPR effect provoked the movement of such NPs, and the process of passive diffusion or transport was along the concentration gradient.

Production of ROS in mosquito larvae

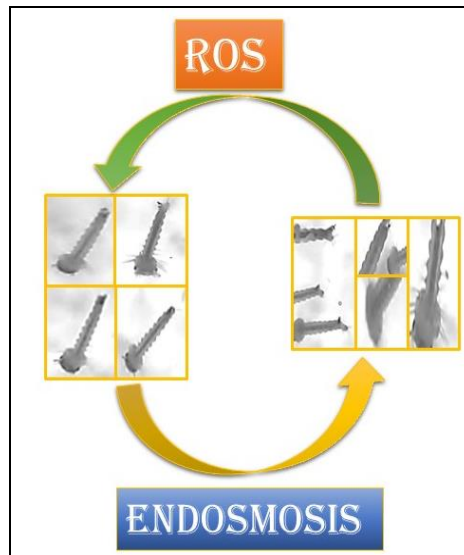


Fig 1: A) Light Microscopic images mechanism of ROS and endosmosis Result In Lysis Of mosquito larvae.

The production of ROS and the action of NPs was a vital phenomenon which marks the unique feature of LgPd NPs. This identity of NPs with little heating effect facilitates the killing impact on mosquito larvae. The production of ROS breaks the lipid bilayer, so there was a formation of leaky structures on the membranes^[12]. This leakage allows the external factors to move inside the larvae. The ROS formation by the LgPd NPs was discussed in detail in the previous data of publication.

Mechanism of endosmosis and lysis of mosquito larvae

Osmosis is the phenomenon by which the liquid moves towards the solute concentration. In brief, the fluid from low concentration moves towards high concentration. Such movement was along the concentration gradient. The endosmosis phenomenon is after ROS formation^[13]. The leaky points were unable to seal by the self mechanism of the cell. Therefore, the fluid outside of the cell environment tries to move inside. This happened due to the loss of equilibrium. With the difference in solute concentration of larvae body and outer atmosphere, the fluid enters the larval structure to balance the stability^[14]. Hence, this osmosis process by which liquid moves inside the larvae is called endosmosis. The endosmosis results in the swelling of the larval body, and finally, the structure will be lysed or burst into pieces. The bulbous swelled forms were identified under the light microscope. ROS promotes endosmosis, and it kills the mosquito larvae^[15].

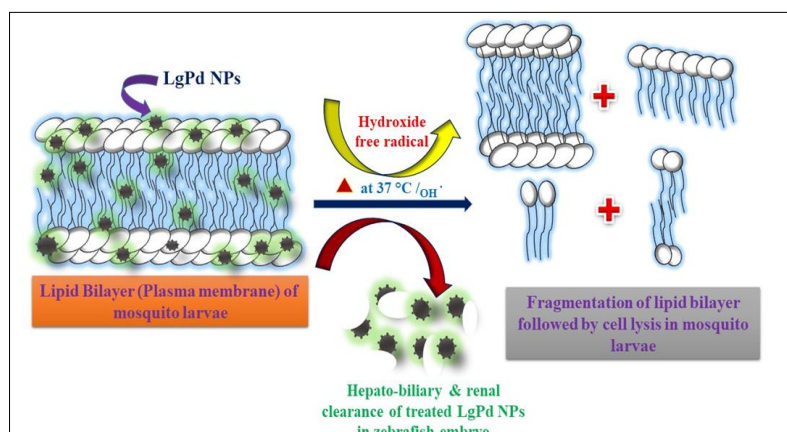


Fig 2: A) The graphical representation of endosmosis mechanism of LgPd NPs in *Culex pipiens* mosquito larvae

The resulting NPs were studied thoroughly for their importance in getting rid of dangerous culex mosquito larvae. These NPs were biocompatible with the higher-order animals, killed the parasites, and became insecticidal and larvicidal in brief. In general, if kept in water, any biological living entity in an unconscious state will become swollen due to the endosmosis process. If such an entity is preserved in water for longer, it initiates the process of damage, and in a few days, the whole structure becomes degraded and will lose its identity. It rotten and decay subsequently ^[16].

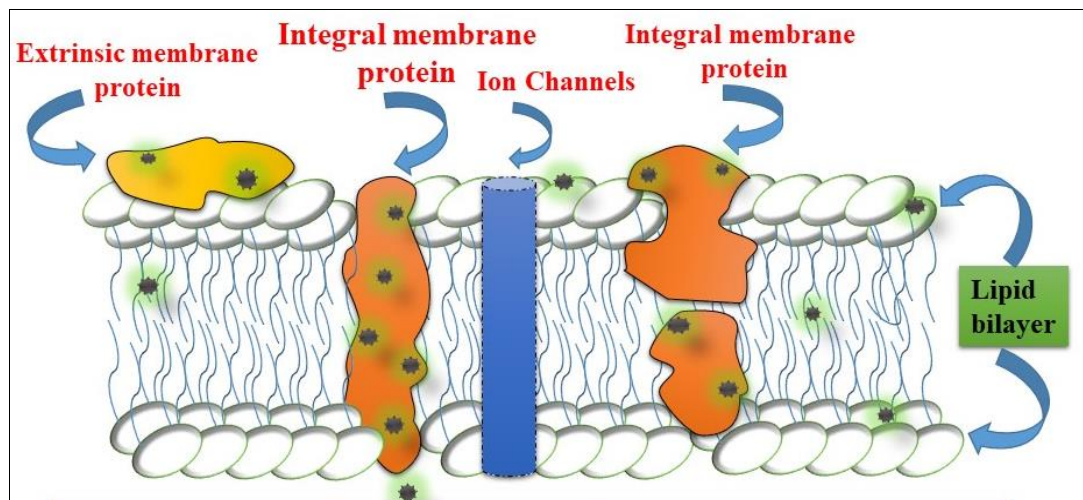


Fig 3: A) Transport mechanism of LgPd NPs Across The cell membrane

Conclusion

In conclusion, it was stated that the prepared NPs become biocompatible with animals except for insecticidal organisms. The ROS and subsequent endosmosis promote the degradation of *Culex pipiens* mosquito larvae in step wise manner. The LgPd NPs enter mosquito larvae's cell, egg, or larval fate via plasma membrane through the channels or the transporters made available to join such particles inside the cell. In final, the ROS promotes the leakage in the membranous envelope, and endosmosis finally bursts the structure altogether. Therefore, the LgPd NPs ultimately proven for use as a larvicidal entity and can be subsequently utilized for clinical approval in future research to explore their real benefits for humanity.

Conflict of interest

All the authors declare no conflict of interest to mention.

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