International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Plant-Derived Edible Vaccines: A Review

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ABSTRACT

For a long time, vaccines have been the main mode of defense and protection against several bacterial, viral, and parasitic diseases. Immunizing humans or animals with edible plants is an evolving tool that seems to grip countless potential. The selected/desired antigen of pathogens (HIV, tuberculosis, etc) are inserted into the selected host plant by transformation technique to form a transgenic plant. Edible vaccines grip countless promise as a profitable, easily managing, can be stored easily, unlikely or unable to fail and sociocultural gladly sustainable, particularly for the poor emerging nations. An edible vaccine takes the place of sore immunization methods. Compare to the conventional vaccine, the edible vaccine is inexpensive, needle-free, eradicates the requirement for preservation, harmless, may be stored nearby to a place of usage, and deliver mucosal and complete immunity.

For enhancing immunity in humans and animals against Various types of infectious diseases such as cholera, measles, FMD, and hepatitis B there are numerous types of edible vaccines are being developed. Edible vaccines also help to overpower autoimmunity diseases alike type-I diabetes. Immunization generations and giving malnutrition have the dual advantages of edible vaccines. If the notable problem and challenges may be triumph over It also can bring about a destiny of more secure and further effective immunization. An edible vaccine is when the antigen is expressed in the edible part of the plant. This reduces the cost of production of the vaccine because of ease of culturing. This article focused on the conception of edible vaccines highlighting the various ways by which vaccines can be delivered.

LITERATURE REVIEW

Edward Jenner:
Edward Jenner is considered the founder of vaccinology in the West in 1796, after he inoculated a 13 year-old-boy with vaccinia virus (cowpox), and demonstrated immunity to smallpox. In 1798, the first smallpox vaccine was developed.

Hiatt and co-workers:
In 1989, the effort to produce a plant-based vaccine was formulated by Hiatt and co-workers.

Dr. Arntzen:
In 1990 Dr. Arntzen introduced the concept of using transgenic plants to produce and deliver subunit vaccines. This idea of Arntzen proved that the edible vaccine can annihilate the restrictions in the production of traditional vaccines.

National Institute of Allergy and Infectious Diseases:
In 1998 approved edible vaccine for its remarkable effect of immunogenicity.

INTRODUCTION

More than one million people die each year of infectious diseases. Fifty percent of these diseases are caused by pathogens infecting the mucosal membrane of the mammalian host. The challenge today is to find unique and innovative vaccines that can target pathogens and infections at various stages. The spread of infectious diseases such as diphtheria, tetanus, polio, measles mumps rubella and hepatitis was reduced by the administration of vaccines. Our immune system destroys disease-causing germs that we call as pathogens and protects our body from their invasion. If our immune system is not strong enough to fight against the invading pathogens definitely we will get infectious diseases. There comes the importance of vaccination.

An antigenic substance prepared from the causative agent of a disease or a synthetic substitute, used to provide immunity against one or several diseases is known as vaccines and the process of administration of vaccines is called vaccination. Vaccines are biological preparations that improve our immunity.

The concept of vaccination was first put forth by Edward Jenner in 1796 for smallpox. Vaccination is the process by which the body is made ready to face and fight off new infections. This way of treatment is in direct contrast to the classical way of treatment, which usually is done after the onset of a specific disease. Vaccines not only prepare us against any future infection but also immunize us against those infections for a very long time. The major drawback until now has been the production process. Vaccines are generally produced by industrial processes, thus making them expensive and inaccessible in developing countries. For this very reason, edible vaccines are seen as ideal replacements for conventional vaccines. Edible vaccines are generally antigen-expressing plants, thus requiring basic knowledge on agriculture and how to grow plants to be produced. Also, in edible vaccines, the process of purification and downstream processing, which make conventional vaccines costly, are eliminated.

CONCEPT OF EDIBLE VACCINES

The idea of edible vaccines was established in the 1990s. The edible vaccine first demo has taken in the tobacco on the mutants of bacterium streptococcus, in the mutants, the surface antigens were expressed. Dental caries are caused by this bacterium, it was predicted that the stimulation of a mucosal immune response could prevent the bacteria from colonizing the tooth and therefore defend in opposition to tooth decay.

Edible vaccines are created by introducing the desired gene into a plant to manufacture the encoded protein. The coat protein of a specific virus or bacteria that has no pathogenicity is used for transformation. The various transformation techniques used for plant, algal, and bacterial vaccine carriers. Edible vaccines can be very easily scaled up. For example, the entire population of India could be vaccinated by producing edible vaccines in just 30 hectares of land. The chance of contamination by plant pathogens is very low or rather insignificant as plant pathogens are not capable of infecting human beings. Edible vaccines against various diseases such as measles, cholera, foot and mouth diseases, and hepatitis B, C, and E are produced in plants like bananas, tobacco, potato, etc.

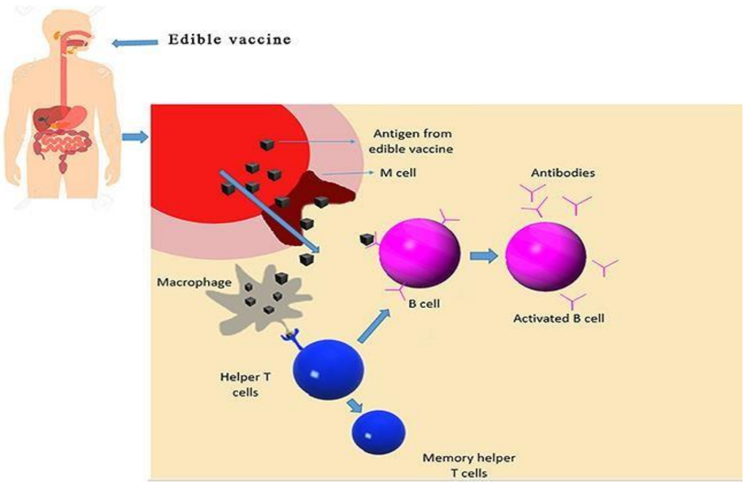
PLANT-DERIVED EDIBLE VACCINES

Edible vaccines are transgenic plants manufacture that contains agents which generate animals' immune responses. Simply, edible vaccines are plant-made pharmaceuticals. Edible vaccines are the preparations of subunit where they are prepared t contain antigens that might be produced in genetically altered plants and supplied to the plant's parts which are able to eat. Transgenic plants in the edible vaccines are used as vaccine production systems. In plants, the genes can be expressed which encoded the antigens of viral and bacterial pathogens wherein they maintain the native immunogenic properties. Antigenic proteins are the composition of Edible vaccines and pathogenic genes are deficient, it works in a comparable manner as the inserted DNA vaccine. Therefore, after the ingestion of edible vaccine it becomes assimilated, and then protein move in the bloodstream, the infectious protein is neutralizing due to the immune response and makes a reminiscence spot of it.

Development of vaccines (edible vaccines) through the transformation process in which selected desired genes are incorporated into plants and then allowing these altered plants to produce the encoded proteins. The plants which are altered through the process of transformation are called transgenic plants. The introduction of selected genes into selected plants through different plants genetics methods such as Electroporation method, Micro projectile bombardment method, Agrobacterium tumefaciens Plasmid vector carrier system. The edible vaccine can additionally motive a destiny of harmless and additional efficient immunization.

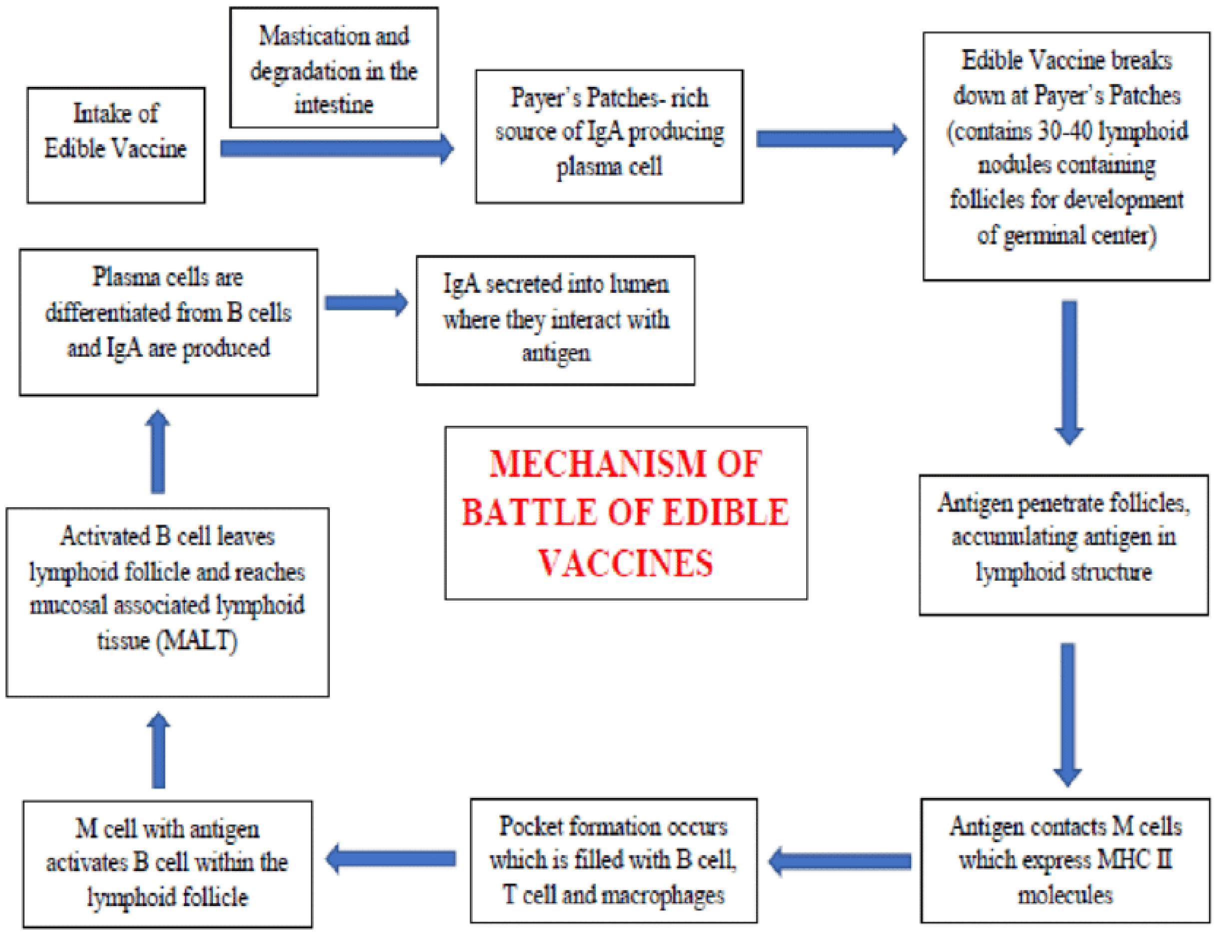
MECHANISM OF ACTION OF EDIBLE VACCINES

Edible vaccines are required to induce the activation of the mucosal immune response system (MIS). The MIS is the first line of defense as it is where human pathogens initiate their infection. Mucosal surfaces are found lining the digestive tract, respiratory tract, and urino-reproductive tract. There are multiple ways by which the antigen can enter the gut mucosal layer, namely by M cells and macrophages. Macrophages are usually activated by interferon-gamma. This activation leads to the macrophages presenting fragmented peptides to the helper T cells that further produce antibodies. M cells are another way by which the antigens are transported to the T cells. The antigenic epitopes are then present on the APC surface with the assistance of helper T cells, which then activate B cells. Activated B cells then migrate to the mesenteric lymph nodes where they mature into plasma cells, which then migrate to mucosal membranes to secrete immunoglobulin A (IgA). IgA then forms the secretory IgA, which is then transported into the lumen. Production of secretory IgA is another complex event since 50% of secretory IgA (sIgA) in gut lumen is produced by B1 cells in the lamina propria in a T-cell-independent fashion. This sIgA are polyreactive and usually recognize foreign antigens. In the lumen, the sIgA neutralizes the invading pathogen by reacting with the specific antigenic epitopes.



The most common problem most oral vaccines/therapeutics face is the tolerance towards the vaccine in the gut. This problem can be overcome by some methods:

Immune suppression by using triamcinolone. However, this has to be done in small amounts so as prevent any major health concerns or even fatalities. Increasing the dosage of the vaccine significantly can often lead to jump-starting the immune response.



THE MOSTLY USED PLANTS FOR EDIBLE VACCINE PRODUCTION

Organs and tissues of different plant species are used for edible vaccine production. A plant for vaccine production must be selected according to some features such as transformation capability, adequate-protein expression in edible tissue, and production of non-toxic compounds for target users. Leaves, fruits, tubers, and seeds are commonly used as edible parts of plants for the expression of recombinant proteins.

Besides field cultivation, plant tissue and organ cultures including main cell and hairy root cultures are biotechnological systems for the high, expression of desired proteins. Seeds are very important for commercial production expressing recombinant proteins at sufficient amounts

- Candidate plants are those plants that are most suitable for edible vaccine production. There are a number of factors that make a plant a good edible vaccine candidate.

1. Must have a long shelf life. The plant or the edible part of the plant has to be stored for a long time without degradation. Cereals such as rice, maize, and wheat are great examples of such plants.
2. Must grow quickly. Fruits or vegetables that usually are produced on trees are considered bad candidates as they take a long time to grow and mature, whereas plants such as tobacco and tomato have a fast growth time.
3. Easy transformation. Plants on which considerable research has been carried out and transformation techniques optimized are very good candidate plants.

Banana:

Banana is the commonly used plant species in the production of edible vaccines. Inexpensive when compared to other plants. Banana plants express HBsAg. The leaf contains antigen.

Alfalfa:

Alfalfa is the plant used to develop edible vaccines mainly for veterinary purposes. Transgenic alfalfa containing hog pest virus glycoprotein E2 was developed in 2005.

Lettuce:

This plant is an effective model system against enteric diseases in both animals and humans caused by E coli. This plant is mainly used up in the raw form and it produces beneficial effects against the hepatitis B virus. It is the utmost effective plant that can be used as an edible vaccine

Carrots:

Carrots were not only healthy and delicious but also can be consumed in the form of edible vaccines. Vaccines against HIV, E coli, Helicobacter pylori shows potential effects when it is produced in transgenic carrots.

Tobacco:

Tobacco is not an edible plant. It is used as a model for the development of edible vaccines. A vaccine was developed in tobacco for the Norwalk virus in 1996 that causes gastroenteritis.

Potato:

Potato is an appropriate model for producing vaccines against tetanus, diphtheria, hepatitis B, and Norwalk virus.

Plant	Advantage	Disadvantage
Tobacco	Easy and efficient to transform. Have high amounts of leaves and seeds as major source of biomass. Do not have a complex protein-lipid content which makes it easy to purify the protein	Can not be consumed orally because of the high alkaloid content.
Potato	Model plant in edible vaccine production. Easy and efficient to transform. Tubers can be eaten and has specific promoters. Clonal propagation allows stable production of transgenic plant lines. Outcrossing risk is very low.	Can not be consumed uncooked.
Tomato	Grows fast. Can be consumed uncooked. Have fruit specific promoters. Easy and efficient to transform. Has a well-defined process in food industry. High content of vitamin A may boost immune response.	Protein content is low in the fruit. Acidic nature of the plant cannot be suitable for all antigens.

Banana	High amounts can be found in Africa where economical vaccines are required. Can be consumed both by children and adults easily. Clonal propagation allows stable production of transgenic plant lines. Outcrossing risk is very low	Protein content is low in the fruit. Needs big farming area to be produced.
Strawberry	Can be consumed uncooked.	Spoils quickly. Requires special storage conditions.
Rice	Due to low allergenic potential it's commonly used in baby food. High amounts of proteins/ antigens expressions	Grows slowly. Can not be consumed uncooked.

PRODUCTION OF EDIBLE VACCINES

Edible vaccines can be produced by the incorporation of the transgene into the selected plant cell. The integration of the transgene can be done without combining with vector by direct gene delivery method or by combining with the vector by indirect gene delivery method. The transgene can be expressed in the plants by two transformation systems depending on the site where antigen should be merged with the cells (stable transformation and transient transformation system)

1. Direct Gene Delivery Method

Direct gene delivery is a simple method. In this, the selected DNA or RNA is directly introduced into the plant cell. The most commonly used direct gene delivery method is the biolistic method and it is also known as gene gun or micro-projectile bombardment method. This is a vector-independent method. This is done when gene transfer through agrobacterium species-mediated transformation is not possible.

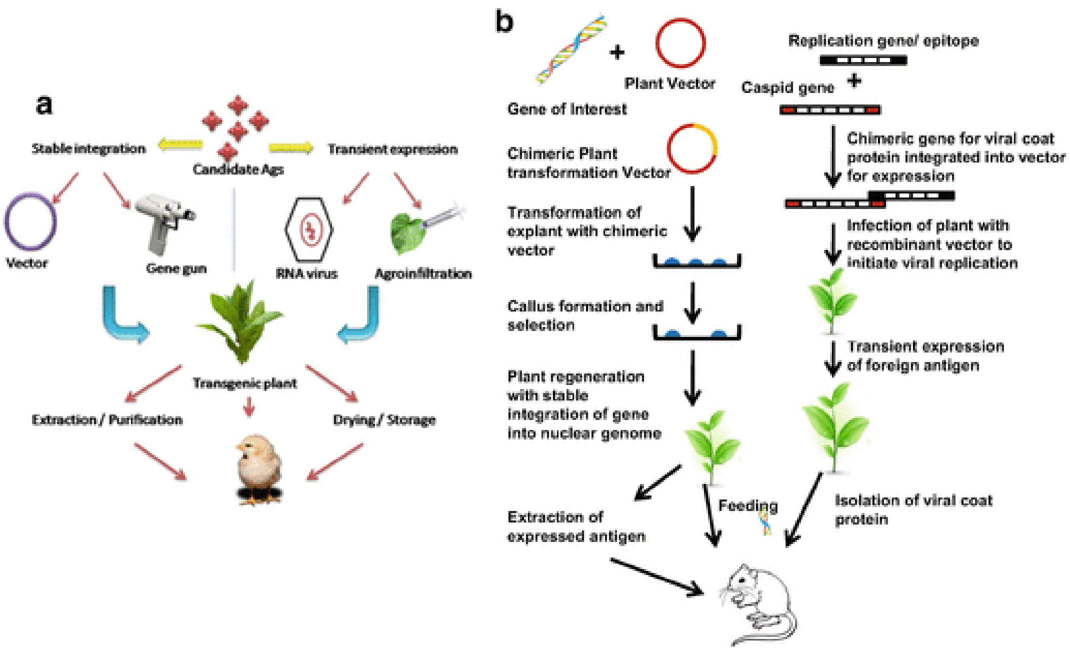
In this transformation method, the DNA or RNA is coated with gold or tungsten which acts as a micro-carrier. Then, the coated DNA is placed into the gene gun and is exposed to high pressure of Helium gas. The coated DNA will move due to high pressure and get penetrated into the targeted plant cell. This method requires a very high cost and can harm the plant.

Transformation can be done by the biolistic method. These were the two types of antigen expression methods.

The most commonly adopted method for the production of edible vaccines is chloroplast transformation.

2. Indirect Gene Delivery

This is a vector-mediated gene delivery. In this method, the desired plant cells were infected with plant-bacteria or plant viruses to produce the protein of interest.



APPLICATIONS

❖ ***Edible Vaccines for Human Use***

Hepatitis B

Hepatitis B is one of the most common viral diseases of humans. Plant-derived vaccines can at least partially replace present hepatitis B vaccines with a lower cost than the regular vaccine.

Hepatitis B surface antigen (HBsAg) is the first antigen expressed in Nicotiana tabacum. Due to the high alkaloid content of the tobacco, potato was used for HBsAg production to be used in oral applications.

Autoimmune Diseases

In the case of autoimmune diseases such as type 1 diabetes, it is very much useful to take self- antigens. Damage of beta cells and fails to produce insulin is the main reason for diabetes.

Potatoes contain insulin or glutamic acid decarboxylase along with innocuous B subunit of the Vibrio cholera toxin shows a better improvement in diabetic mice. It could supress the immune responses and maintains the level of insulin.

Plant derived therapeutics on market/approved

Aim	Plants	Product	Status
Newcastle disease	Tobacco [Suspension culture]	HN antigen of Newcastle disease	USDA approved
Dental caries	Tobacco	CaroRX	EU approved
Vaccine Purification	Tobacco	Antibody against Hepatitis B	On market
Gaucher's disease	Carrot [Suspension culture]	Elelyso [Taliglucerase alfa]	USDA approved
Human growth factor	Barley	ISOkine™ , DERMOkine™	On market
Vitamin B12 deficiency	Arabidopsis	Human intrinsic factor	On market
Anti-infection, anti-inflammatory	Rice	Human lactoferrin	On market
		Human lysozyme	

ADVANTAGES AND DISADVANTAGES OF PLANT-DERIVED EDIBLE VACCINES

Advantages:

- Have low cost, easy administration and highly scalable features.
- Can be one of the alternatives of the traditional vaccines as they can overcome all the problems encountered with traditional vaccination against infectious diseases, autoimmune diseases and tumours.
- Can be used as raw food or dry powder.
- Are targeted to elicit both mucosal and systemic immunity although in
- traditional vaccines mucosal immunity is not obtained.
- No need for "Cold chain" that results in low cost of storage, preparation, production and transportation.
- Stable at room temperature unlike traditional vaccines.
- Manufacturing cost is lower than traditional vaccines costs because no sterilization is needed.
- Could be the source for new vaccines combining numerous antigens [second generation vaccines].
- Do not involve attenuated pathogens and there is no risk of proteins to reform into infectious organism.
- Mass production is easier compared to an animal system.

Disadvantages:

- Degradation of protein components in the stomach and gut.
- The best plant species or tissue for commercial production is not clear.
- Levels of pharmaceutical proteins produced in most transgenic plants are not adequate for total immunity and commercial feasibility.
- There are several successful reports of high-level expression of non-human proteins via the nuclear genome, but it is needed to increase expression levels of proteins to enable the commercial production of pharmacologically important proteins in plants.
- Effective dosage requirement is not clear.
- Consistency of dosage and stability differs from plant to plant, fruit to fruit, generation to generation according to protein content and patient's age.
- The quantity of plant tissue constituting a vaccine dose must be at optimum size for consumption.
- They need to be stored in optimum conditions to prevent microbial spoilage.
- Short storage life and long production cycle make difficult vaccine production in some plants like tomatoes and banana.
- It is expensive to extract the desired protein due to the presence of interfering compounds in plants.
- Commercial and economic successes are dependent on the protein amounts produced by plants.

CHALLENGES

There were specific issues related to edible vaccine. Plant-derived vaccines should be clinically tested under US investigational new drug application, and also must follow all the regulatory and GMP requirements.

The future of edible vaccine depends on many criteria. It should be well approved by the population so that it is necessary to make aware the society on the use and benefits of edible vaccines. In some areas, it is believed that genetically modified plant and products were a threat like evil spirits and destroy the world so there is a crucial role to awake the people from this myth of evil spirit by the authorities. The next important benchmark to check is the stability of the genetically modified plants and proper isolation of the plant is essential. Sometimes the transgene causes allergies. Plant-made oral vaccines might induce allergic reactions during post-translational modifications, and oral tolerance when co-administered with oral adjuvants to mostly activate the mucosal immune system may provoke hypersensitive responses to other proteins contained in the daily food.

Growing plants for edible vaccine production requires close monitoring. The safety and quality of the genetically modified plants will be a difficult task even though manufacturing of genetically modified plants are regulated. Cross-contamination between genetically modified plants and non-genetically modified plants may occur during pollination and the genetically modified plants.

CONCLUSION

Plant-based vaccines are the emerging type of vaccines that have a higher therapeutic value to treat many human and animal diseases. A stable and transient gene expression can be obtained based on the gene delivery methods used. By far, chloroplast transformation via biolistic or particle bombardment gene delivery method has been considered as a very promising alternative for better production of plant-based vaccines. However, the development and improvement of suitable gene delivery methods for efficient and optimum vaccine production shall be continued.

There are also some bioethical issues arising from the production of plant-based vaccines such as the risk of transferring allergens from transgenic plants to humans and animals. As some of the plant-based vaccines use bacteria and viruses as vectors, the pathogens might be reactivated and infect other organisms that consume them. The benefits and advantages of plant-based vaccines shall be able to overwhelm the challenges faced by this interesting biological product. Thus, it is anticipated that regulatory approval will be granted ultimately to help in global disease control.

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