https://doi.org/10.48047/AFJBS.6.14.2024.2200-2222

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

EDIBLE VACCINES- INNOVATIVE APPROACH FOR ORAL IMMUNIZATION

Dr. Manasi Wagdarikar*[1], Dr. Anagha Joshi[2], Dr. Dayanand Kannur[3], Mrs. Pallavi Chouhan[4], Ms. Manasi Jadhav[5], Ms. Vaishnavi Madavi[5], Ms. Tanvi Salunke[5], Mr. Nishant Mali[5], Ms. Shraddha Ingawale[5], Mr. Kartik Yenkure[5], Mr. Prashant Jadhav[5]

1. Associate Professor, Department of Pharmaceutical Chemistry, SCES's Indira College of Pharmacy, Pune, Maharashtra.
2. Professor, Department of Pharmaceutical Chemistry, SCES's Indira College of Pharmacy, Pune, Maharashtra.
3. Professor, Department of Pharmacognosy, SCES's Indira College of Pharmacy, Pune, Maharashtra.
4. Assistant Professor, Department of Pharmaceutical Chemistry, SCES's Indira College of Pharmacy, Pune, Maharashtra.
5. Research Scholar, Pharmaceutical Quality Assurance, SCES's Indira College of Pharmacy, Pune, Maharashtra.

***Corresponding Author**: Dr. Manasi Wagdarikar, **e-mail**: mbkulkarni84@gmail.com

Article History

Volume 6, Issue 14, Aug 2024

Received: 15 July 2024

Accepted: 7 August 2024

Published: 8 August 2024

doi: 10.48047/AFJBS.6.14.2024.2200-2222

ABSTRACT:

Vaccines represent a useful contribution to the branch of biotechnology as they provide protection against various diseases. However, the major hurdle to oral immunization is the digestion of macromolecule antigenic protein within the stomach due to extremely acidic pH. To address this issue, scientist Arntzen developed the theory of edible vaccines (EVs). EVs are developed using the genetic engineering technology in which the appropriate genes are introduced into the plants using various methods. This genetically modified plant then produces the encoded protein which acts as a vaccine. Owing to its low cost, it will be affordable for developing countries like India. EVs are developed to treat various diseases such as malaria, measles, hepatitis B, stopping autoimmunity in type-1 diabetes, cholera, enterotoxigenic Escherichia coli (ETEC), HIV, and anthrax. Even though they have some disadvantages like control of dosage of the antigen that is present in the recombinant fruit or vegetable, they have many advantages as they trigger the immunity at mucosal surfaces which is the bodies first line defense. Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, fail-safe and sociocultural readily acceptable vaccine delivery system, especially for the poor developing countries.

BACKGROUND:

In order to control a number of diseases, vaccinations are essential. Conventional vaccines are being widely used in the world but their production requires higher cost and more time. Growing world population and continuous disease emergence have invited the development of better infrastructure. Thus, the idea of plant-based edible vaccine technology has emerged and showed promising results with strong and effective protection against many diseases. Plants have been utilized since more than two decades as pharmaceuticals against many diseases. Methods: Plant-based technology has great potential to express genes and produce clinically important compounds in the desired tissue. Plant biotechnology has played important role in the production of pharmaceutical compounds like vaccines, antibodies, antigens, sub-units, growth hormones and enzymes by utilizing genetic modification. It has also opened a new approach for developing an edible vaccine as an oral delivery. Currently, many pharmaceutical proteins have been developed as edible vaccine in different plant expression systems and evaluated against various life-threatening diseases and some of them have reached advanced phase of the clinical trial and exhibited promising results. (1,2,3)

OBJECTIVES OF EDIBLE VACCINE:

Plant-based edible vaccines represent an innovative approach in vaccine technology, leveraging plants to produce antigen proteins that can induce immune responses when consumed orally. This technology offers several potential advantages over conventional injection vaccines:

1. Ease of Administration: Edible vaccines can be incorporated into food, making them easy to administer, especially for children who may resist injections.

2. Mucosal Immune Response: Unlike injection vaccines, plant-based edible vaccines have the potential to stimulate mucosal immune responses in the gastrointestinal tract. This is particularly advantageous for developing vaccines against gastrointestinal infectious diseases.

3. Elimination of Cold Chain Requirement: Traditional vaccines often require refrigeration throughout the distribution chain to maintain efficacy. Plant-based vaccines, however, may not

need a cold chain, which simplifies storage and distribution, especially in regions with limited infrastructure.

4. Cost-Effectiveness: Production costs for traditional vaccines can be significant, partly due to the need for specialized manufacturing facilities and cold chain logistics. Plant-based vaccines could offer a more cost-effective alternative, potentially reducing overall vaccine costs.

Despite these promising attributes, it's important to note that there are currently no commercially available plant-based edible vaccines on the market. Research and development in this field are ongoing, aiming to address regulatory, safety, and efficacy challenges associated with this novel vaccine delivery method.

In summary, while plant-based edible vaccines hold great potential for revolutionizing vaccine delivery, particularly in resource-limited settings, further research and development are needed to bring these innovations from the laboratory to widespread clinical use.(4,5,6)

INTRODUCTION TO VACCINE:

Vaccines come under the branch of biotechnology that provides defense against a range of infectious diseases and disorders. Throughout their lives, all organisms are susceptible to one or more infectious or non-infectious disease types. Researchers have developed a plant-based vaccine, an immune biological material that provides targeted defense against viral and non-infectious diseases, in an effort to stop these infections. vaccination is the process of administrating and dispersing vaccines, and vaccination is a type of immunization. (7,8,9,10)

Even though children are immunized against the six deadly illnesses all across the world, 20% of newborns remain unvaccinated, accounting for about two million avoidable deaths occur each year, primarily in the world's most isolated and underdeveloped regions. The limitations on vaccine production, distribution, and delivery are the reason for this.(11)

Vaccines can be therapeutic or preventive, depending on the situation. In addition to preventive vaccines, which prevent infections, therapeutic vaccines are also being researched and may help avoid the complications of chronic illnesses including hepatitis B, HIV, and HPV by strengthening the immune system. The three primary approaches for producing vaccinations are cell-based vaccine, vaccines created using an experimental manufacturing technology, and vaccines made in eggs.(12)

Vaccine administration involves activation of the immune system against a specific disease, readying it for potential invasion. Immunizations are administered for active vaccination as a preventative strategy against certain infectious diseases. For several months or years, they offer full or partial protection. Immunizations can be administered orally, nasally, or parenterally by injection techniques such intramuscular (IM), subcutaneous (SC), and intradermal (ID). It is widely known that the sort of immune response can be affected by the delivery route. Most commercial vaccinations are delivered by SC or IM methods.(13)

INTRODUCTION TO EDIBLE VACCINE:

In the 1990s, Arntzen explored the idea of edible vaccines. The first instance of an edible vaccine was demonstrated by a tobacco-derived surface antigen from the bacteria Streptococcus mutans(14).The process of creating edible vaccines involves introducing the required genes into plants, which causes the required encoded proteins to be produced. This method is referred to as "transformation," with the altered plants referred to as "transgenic plants." The majority of edible vaccinations are genetically modified (GM) crops that boost resistance to certain illnesses including HIV, Hepatitis B, diarrhea, pneumonia, and so forth. Vaccines that are edible have pathogenic proteins but no infectious genes. There is no proof that consuming vaccines causes sickness, and immunity is certain.(15) They can be kept close to the location of usage, are less expensive, heat-stable, and do not require cold-chain maintenance. No need of needles or syringes, they demonstrated good genetic stability and were produced locally utilizing conventional techniques and don't need expensive facilities for producing pharmaceuticals.(16) The concern with whole fruit or vegetable vaccines is the consistency of dosage from fruit to fruit, plant to plant and generation to generation. And limitation is storage of edible vaccines.(17)

Since plant viruses cannot infect humans, one significant benefit of edible vaccines is the removal of animal virus contamination, such as the mad cow disease risk associated with vaccinations made from cultivated mammalian cells. As soon as edible vaccines come into contact with the lining of the digestive tract, they begin to stimulate both systemic and mucosal immunity. Due to their dual mode of action, edible vaccines offer first-line protection against infections that assault the mucosa, such as Mycobacterium tuberculosis and its carriers, which can result in HIV, STDs, diarrhea, and pneumonia.(18)

Oral delivery of palatable vaccinations to mothers may be helpful in immunizing the fetus in pregnancy through the transfer of maternal antibodies across the placenta or the child through nursing. The process of seroconversion in the presence of maternal antibodies is made feasible

by edible vaccines, which may help shield kids from illnesses like group-B streptococcus and respiratory syncytial virus (RSV), among others.

Currently, there are edible vaccines available for a number of diseases that affect both humans and animals, including cholera, foot and mouth disease, measles, and hepatitis B, C, and E. They can also be used in conjunction with other vaccination programs to provide multiple antigen distribution, thereby preventing rare diseases including rabies, dengue, and hookworm. A range of vegetables, such as rice, lettuce, bananas, potatoes, and tomatoes, are being studied for potential use in edible vaccines.(19)

DIFFERENCE BETWEEN TRADITIONAL VACCINE AND EDIBLE VACCINE (20,21,22,23,24)

Traditional vaccine	Edible vaccine
• Too expensive to be used on large scale. • Lack of physical infrastructure (roads and refrigeration) makes it impossible to disseminate the vaccine. • Require trained personnel to administer injection. • Require elaborate production facilities, purification, packaging, or specialized delivery system. • Can not directly stimulate the immune system.	• Comparatively less expensive if produced in large amount. • May be easily available. • Do not require any trained personnel to administer. • No purification strategies are required. • Vaccine when taken orally, can directly stimulate the immune system.

DIFFICULTIES IN TRADITIONAL VACCINE:

Conventional vaccinations are primarily limited by their storage, transportation under closely monitored circumstances (which requires a cold chain system), and potential for negative reactions resulting from improper handling or inadvertent inoculation.

Standard oral vaccine system requirements include:

- Sufficient amounts of desired antigens should be present.
- Long-term room temperature stability of expressed antigen
- The vaccination must induce protective immunity.
- Able to tolerate stomach enzyme breakdown. (25,26,27)

PLANT BASED VACCINE:

A literature search for plant-made antigens or vaccines yields several hits that describe the expression of various vaccination antigens in a variety of plant systems. The efficiency and effectiveness of the generated proteins, rather than the capacity of plant systems to express antigens, are what need to be shown to the larger protein production community. In addition, the systems must receive regulatory and safety approval and show that they can produce goods profitably on a wide scale.

When it came to the amount of recombinant protein produced and production time, the main recognized drawback of the plant-made recombinant protein platform was its incapacity to compete with other recombinant protein production platforms. Plants produced at least 10 times less of the desired protein than other platforms like bacteria or animal cells, and it could take up to 18 months to create a stable transformed plant line. However, with the advancement of temporary plant transformation systems, this circumstance is no longer applicable. Plant virus-based expression vectors that are small and deconstructed can be delivered using agroinfiltration. (28)

Through his demonstration of plants' ability to function as quick response production systems, D'Aoust made a significant advancement in the field of plant-based vaccines. Nicotiana benthamiana was used as a test subject for a transient expression system that enabled the transient expression of protective antigens from various influenza strains. Large volumes of protective antigen (50 mg/kg) against the H5N1 (AIV) and H1N1 (human) strains were produced by the agro-infiltrated plants. The important thing to note, though, was that the entire process took less than three weeks, from the release of the viral genome to the finished vaccine. Therefore, this quick approach for producing vaccines might be helpful in preventing disease outbreaks and facilitating the prompt mobilization of staff. (29,30)

There are several plant-based platforms available for producing target antigens of interest, such as complete plants, organs, or cells, as well as expression technologies. (31,32) Potato, tomato,

and tobacco are representative plant species that express the oral vaccine; maize, rice, carrot, and soybean are also used in this field.(33,34,35,36,37,38,39,40,41)

These plants are primarily concentrated on conventional crops that humans often consume, as it is well known that immature plants might occasionally suffer from specific plant allergies.

Plant components like as fruits, seeds, and root vegetables serve as the basis for edible plant vaccinations. Food vaccines of this type are made without the need for costly antigen purification, which is a need for parenteral vaccination delivery. (42)

Thus, the lyophilization of organs expressing stable antigens might enable more affordable and useful vaccines by facilitating their processing, purification, and storage. The low production level of the resulting recombinant protein is still a cause for worry, despite the widespread acceptance of stable transformation into transgenic plants. For vaccine antigens and other therapeutic proteins, plastid transformation is a useful substitute for nuclear transformation.

Chloroplast transformation has been shown to have the highest transgenic expression, accounting for up to 70% of total soluble protein. Otherwise, 50 µg/g of fresh leaf tissue or 1% of total soluble protein (TSP) has been the universal expression threshold in the majority of investigations. Additionally, transgenic containment debate can be avoided by using chloroplast technology to express several genes as a single gene. (43)

IDEAL PROPERTIES OF VACCINE:

- It should be safe and not toxic or pathogenic.
- It should have very little adverse effects in healthy people.
- People with compromised immune systems shouldn't experience issues with it.
- Long-lasting humoral and cellular immunization should result from it.
- Simple immunization procedures should be used.
- The vaccination technique should be simple
- The environment shouldn't be contaminated.
- It need to be affordable and efficient. (44,45,46)

ADVANTAGES OF EDIBLE VACCINES:

- A vaccination that is edible can be consumed like fruits and vegetables.
- Dried leaf tissue powder can be used to make capsules.
- Adjuvants are not necessary to boost immunological responses.

- Mucosal immunity is induced by oral antigens.
- Producing in large quantities on site, transporting, and storing it at a reduced cost without refrigeration is simple.
- No need for an injection and a qualified medical professional.
- The protein is easily expressed, separated, and purified.
- They don't need to be refrigerated when stored as seeds, oils, or dried tissue.
- There is no chance of contamination or the transmission of disease.
- There's a chance for improved compliance, particularly with children. (47,48)

DISADVANTAGES:

- Consistency of dosage from fruit to fruit, plant to plant, lot to lot, and generation to generation is not similar
- Stability of vaccine in fruit is not known.
- Evaluation of dosage requirement is tedious.
- Selection of best plant is difficult.
- Certain foods like potatoes are generally not eaten raw and cooking the food might weaken the medicine present in it.
- Not convenient for infants as they might spit it, eat a part or eat it all, and throw it up later. Concentrating the vaccine into a teaspoon of baby food may be more practical than administering it in a whole fruit.
- There is always possibility of side effects due to the interaction between the vaccine and the vehicle.
- People could ingest too much of the vaccine, which could be toxic, or too little, which could lead to disease outbreaks among populations believed to be immune.
- A concern with oral vaccines is the degradation of protein components in the stomach due to low pH and gastric enzymes. However, the degradation can be compensated by repeating the exposure of the antigen until immunological tolerance is accomplished.
- Potential risk of spreading the disease by edible vaccine delivery is a concern of many. Potential contamination of the oral delivery system is a possible danger. (49,50)

LIMITATIONS AND TECHNIQUES TO OVERCOME LIMITATIONS:

1. Protein Expression:

- Limitation: Initial strategies resulted in poor protein expression.
- Developed Alternatives: New strategies like transplastomic technologies and viral vectors have significantly improved yields.

2. Stability and Dosage:

- Limitation: Unprocessed plant tissues were unstable, making accurate dosage difficult.
- Developed Alternatives: Freeze-dried biomass and seed-based approaches now provide stability and straightforward dosage using powdered seeds.

3. Immunogenicity:

- Limitation: Early vaccines often induced oral tolerance rather than immune responses.
- Developed Alternatives: Use of adjuvants and bioencapsulation in plant cells have improved immune response induction.

4. Good Manufacturing Practices (GMP):

- Limitation: Initial focus did not ensure production under GMPs.
- Developed Alternatives: Implementation of GMP procedures including bioreactor culture and transient expression systems.

5. Biosafety:

- Limitation: Concerns about gene flow contaminating the food chain.
- Developed Alternatives: Transplastomic approaches minimize gene flow risks, and containment strategies in bioreactors ensure biosafety. (51)

MECHANISM OF ACTION:

An innovative method of oral immunization is the edible vaccine. This technology could have a significant impact on health care in developing nations and help the global vaccination effort. Since mucosal surfaces are attacked by pathogens, this is the best place to administer a vaccination. Due to their contact with the lining of the digestive tract, edible vaccines stimulate

both mucosal and systemic immunity. First-line defense against infections that penetrate the mucosa would be provided by this dual action.

The mechanism of action for edible vaccines begins with their intake, followed by mastication and degradation in the intestine. The vaccine reaches Payer's Patches, which are rich in IgA-producing plasma cells. Here, the vaccine breaks down, and antigens penetrate follicles, accumulating in the lymphoid structure. The antigens then contact M cells that express MHC II molecules, leading to the formation of pockets filled with B cells, T cells, and macrophages. M cells with antigens activate B cells within the lymphoid follicle. These activated B cells leave the lymphoid follicle and reach mucosal-associated lymphoid tissue (MALT). Plasma cells differentiate from B cells, producing IgA, which is secreted into the lumen to interact with the antigen.

Mucosal-targeted vaccines, such as edible vaccines, have the ability to stimulate the immune system on the mucosa as well as the systemic level. Measles, cholera, hepatitis B, and many other diseases are being converted into edible vaccines, and many more are in the development stage. Antigenic proteins used in edible vaccines are genetically modified to produce a food product, such as fruits, vegetables, or leafy greens. These eatable goods use certain pathogens that cause disease to contain the protein. After this edible crop is eaten by humans and digested, some of the proteins are released into the bloodstream. The body uses particular immune responses to combat disease-causing pathogens once the bloodstream is sufficiently supplied with these proteins (52,53,54)

DEVELOPING AN EDIBLE VACCINE:

There are two approaches of dealing with genes that encode antigen from known pathogenic organisms (bacteria, viruses, or parasites) for which antibodies are available. In one instance, the full structural gene is placed between the 5' and 3' regulatory elements of a plant transformation vector, enabling transcription and the build-up of coding sequence in the plant. When epitopes within the antigen are found, a DNA fragment encoding them can be fused with a plant virus coat protein gene to create new genes. And the second possibility is, Stabilized plants are then infected with the recombinant virus. The resulting edible plant vaccine is used in additional immunological research.

METHODS FOR PREPARATION OF EDIBLE VACCINE:

Biolistic (Micro projectile bombardment)/gene gun method-

Direct gene delivery is the simplest approach. In this method, the plant cell is immediately exposed to the chosen DNA or RNA. The biolistic approach, also referred to as the gene gun or micro-projectile bombardment method, is the most widely utilized direct gene delivery technique. This approach is independent of vectors. When agrobacterium species-mediated transformation is not an option for gene transfer, this is what is done. (56)

This transformation technique coats the DNA or RNA with tungsten or gold, which functions as a micro-carrier. The coated DNA is then subjected to high pressure helium gas inside the gene gun. High pressure causes the coated DNA to migrate and enter the targeted plant cell. This technique can be harmful to the plant and is quite expensive. (57)

The biolistic approach can be used to perform both nuclear and chloroplast transformations.

Nuclear transformation refers to introducing a desired gene into the plant cell's nucleus through non-homologous recombination, while chloroplast transformation refers to introducing the gene into the chloroplast to boost protein expression. Chloroplast transformation is the most widely used technique for producing edible vaccines. (58,59)

Agrobacterium Tumefaciens:

One method of producing extracellular vesicle (EVs) relies on the type of microorganism that may transfer the genetic instructions for an infectious agent or microbe "antigens," which are proteins that trigger a specific immune response in the recipient, into plant cells [13]. The soil contains Agrobacterium. tumefaciens, which is used in a process known as transformation to introduce a little amount of DNA into plants. One plant cell can regenerate into the entire plant. It has been observed that oral administration of genes that exhibit effective expression in experimental model plants results in the production of serum antibodies in animals. Agrobacterium rhizogenic and Agrobacterium tumefaciens, two vegetable pathogens, have the ability to incorporate their DNA (T-DNA) into the nuclear genome of the infected cell. The research of genes' stable integration into the plant's genome and the creation of a transgenic protein that functions as an EV were made possible by introducing exogenous genes into the suitably modified T-DNA of Agrobacterium cells and then infecting a vegetable tissue. (60,61)

Chimeric virus method:

In order to infect their natural hosts, such as edible plants, where the cloned genes are produced to variable degrees in different edible portions of the plant, plant viruses are genetically altered to

contain the required genes (Fig. 4). Some viruses, like the tomato bushy stunt virus, the cowpea mosaic virus, the tobacco mosaic virus, the cauliflower mosaic virus (CaMV), the potato virus, and the alfalfa mosaic virus, can be engineered to express pieces of antigenic proteins on their surfaces. (62,63)

Electroporation:

Here, DNA is introduced into the cell by briefly subjecting it to a high-voltage electrical pulse; this is supposed to cause temporary holes to form in the plasma membrane.22 Because the cell wall acts as a strong barrier to DNA, DNA must be able to get through it into the cytoplasm of the cell by mildly digesting it. (64)

CHALLENGES:

Before creating a vaccine based on plants, a number of concerns must be addressed. Plant-based vaccines are capable of producing antigen doses that are sufficient, as demonstrated by three efficacious human clinical trials.1719 The individual's weight, age, fruit or plant size, maturity, and protein content must all be taken into account when calculating the appropriate dosage. It's important to know how much to consume, especially for babies who may spit it out, only eat a portion of it, or finish it all and throw up later. A dosage that is too high would result in tolerance, whereas a dosage that is too low would not produce antibodies. It could be more feasible to concentrate the vaccination into a teaspoon of baby food rather than give it as a full fruit. It is also possible to turn the modified plants into tablets, puddings, chips, and other foods. Pureness, dose homogeneity, and lot-to-lot consistency are regulatory considerations. (65)

The main cause of the dearth of research in this area is the concentration of edible vaccine production in developing nations, where larger corporations are more interested in the cattle market than in human application, and smaller groups are funding this study. Additionally, very few local and international government agencies provide support, and those that do are typically underfunded. Due to the lack of funding, grants, research assistance, and investor confidence, many organizations have lost interest in the study of edible vaccines. Additionally, since recombinant vaccines are now so inexpensive, there is less chance to produce edible vaccinations for illnesses like tetanus, diphtheria, etc. when there are already injectable vaccines available. (19)

MOST USED PLANT SPECIES USED AS VACCINE:

Potato:

The potato is a useful model for the development of vaccines against the Norwalk virus, tetanus, diphtheria, and hepatitis B. Potatoes may help make hepatitis B vaccines more palatable for humans. The ease with which potatoes may be modified and multiplied is the main benefit of using them to create edible vaccinations. Refrigerators are not necessary for storing goods, and one of their main drawbacks is that heat denatures antigens. (66, 67)

Tomato:

Tomatoes were initially utilized to develop an efficient vaccine against coronavirus-induced acute respiratory syndrome, or SARS. Compared to vaccines made from potatoes, it has a stronger effect against the Norwalk virus. The Vibrio cholera B toxin's CT-B proteins can be expressed in leaves, stems, fruits, and other tissues. (68)

Tobacco:

The first documentation of the synthesis of an edible vaccine (a Streptococcus surface protein) in tobacco, at a level of 0.02% of total leaf protein, was reported in 1990 as a patent application under the International Patent Cooperation Treaty. Many attempts were then undertaken to express different antigens in plants (Table 9.1). An attempt was made to create an edible vaccine by expressing heat-labile enterotoxin (LT-B) in tobacco, since acute watery diarrhea is caused by enterotoxigenic Escherichia coli and Vibrio cholerae, which colonize the small intestine and produce one or more enterotoxins. (69, 70)

Rice:

The other plant species utilized in the creation of edible vaccines is rice. Benefits over other plants included strong antigen expression and widespread usage in baby feeding. However, it develops slowly and needs a glasshouse environment. A 2007 study using transgenic rice (Oryza sativa) demonstrated a considerable increase in antibodies against E. coli. 2008 saw the confirmation of HBsAg's functional expression in rice seeds. In regions where rice is the primary food source, vaccines made from the plant will have a significant impact on public health. (71,72)

Carrots:

In addition to being wholesome and delicious, carrots can be utilized to create vaccines that are edible. Vaccines against HIV, Helicobacter pylori, and E. coli show possible effects when produced in transgenic carrots. Those with compromised immune systems benefit from consuming this type of consumable vaccine made of carrots and antigens. (73, 74, 75)

Banana:

The most widely employed plant species in the creation of edible vaccines is the banana. It doesn't require cooking. Even when cooked, proteins remained intact. Cheap in comparison to other plants. HBsAg is expressed by banana plants. Antigen is present in the leaf. The primary drawback is that it ripens slowly and degrades quickly after two to three years. (76)

Maize:

A protein produced by maize plants is utilized to create the vaccine against the hepatitis B virus. It doesn't require refrigeration and is less expensive. not need a trained individual or needles to administer the vaccination. Their drawbacks are that they take longer to reach and require cooking to use. (77)

APPLICATIONS OF EDIBLE VACCINE:

Malaria:

Three antigens—merozoite surface protein (MSP) 4 and 5 from Plasmodium falciparum and MSP4/5 from Plasmodium yoelii—are being studied in relation to the generation of EVs. Wang and colleagues have demonstrated that recombinant MSP 4, MSP 4/5, and MSP1, when given orally to mice along with cholera toxin B (CTB) as a mucosal adjuvant, elicited efficient antibody responses against the blood-stage parasite. It has been proposed that low levels of antigen expression in plants mean that high doses of plant material are needed to produce the necessary immunity. Furthermore, a strong adjuvant will probably be needed as well because of the large degree of antigen that is expected to be required. (78)

Hepatitis B Virus:

A transgenic potato plant expressing HBs Ag was used to investigate the antibody-producing ability of mice. Transgenic tomato plants express the major surface antigen of the hepatitis B

virus. Brazil showed the use of transgenic lettuce plants expressing the recombinant HBs Ag antigen of the hepatitis B virus. (79, 80, 81)

Stopping Autoimmunity:

Researchers have discovered a number of data cell proteins that can trigger autoimmunity in individuals at risk for type 1 diabetes. They have also attempted to develop a plant-based diabetes vaccine in potato and tobacco plants that contain insulin or GAD linked to the harmless B subunits of the V. cholerae toxin to improve the uptake of antigens by M-cells. Furthermore, they have developed transgenic potato and tobacco plants that, when fed to non-obese diabetic mice, showed increased levels of IgG, an antibody linked to cytokines that suppress harmful immune response. Finally, feeding the vaccines to a strain of mice that develops diabetes helped to suppress the autoimmune attack and prevents the delay of high blood sugar. (82)

Norwalk virus:

When given transgenic potatoes expressing the Norwalk virus antigen, nineteen (95%) out of twenty individuals showed seroconversion. To tackle the Norwalk virus, genetically modified bananas and powdered tomatoes expressing the virus are now being developed. (83)

HIV:

Two HIV protein genes were injected with a needle along with promoters like CaMV to create genetically modified tomatoes. The expressed protein was detected by polymerase chain reaction in various plant sections, including the ripe fruit and the second-generation plant. It has been possible to successfully inoculate spinach with Tat protein expression cloned into TMV recently. Spinach leaf tissue was found to have 300–500 mg of Tat antigen per gram. When mice were fed this spinach, higher antibody titers were seen compared with the controls. (84, 85)

Anthrax:

When the pag gene (Anthrax protection antigen, or PA) was introduced into tobacco leaves via a gene gun, a protein that is structurally similar to the main protein in the current vaccine was expressed. It is possible to synthesize anthrax antigen in billions of units. Furthermore, the fatal

and edema factors in this vaccination, which cause harmful side effects, were absent. Tomato plants are now being injected with the same anthrax antigen. (86)

Cholera:

It has been shown that transgenic potatoes with the Vibrio cholerae CT-B gene work well in mice. It was said that immunity may be obtained by consuming one potato per week for a month, along with sporadic boosters. It has been demonstrated that nasal delivery of mutant cholera toxin subunit A (mCT-A) and LT-B in agricultural seed is both successful and practicable. (87, 88)

FUTURE OF EDIBLE VACCINES:

Since there are still some concerns about the use of edible vaccinations, much work needs to be done in the future to ensure their safe usage. Because of the concerns expressed, the public's approval of these edible vaccinations is the primary goal, as these genetically modified products negatively impact both society and the environment. When these plants are being grown for edible vaccines, extreme caution must be used to prevent cross-contamination between genetically modified and non-genetically modified plants. Incorrectly, these can also enter the food chain that feeds humans.

However, because they are inexpensive, simple to administer, and only need minimal storage conditions, these edible vaccines have a very high potential in underdeveloped nations. Its original goal was to prevent infectious diseases, but it has now shown promise in the treatment of cancer and other autoimmune illnesses. The concept of genetically modified crops is being promoted by both industrialized and developing nations. It will be determined by future research and development if edible vaccines can satisfy WHO quality requirements for safety, potency, efficacy, and purity. (89, 90)

REFERENCES:

1. Hilleman, M. R. (2003). Overview of the needs and realities for developing new and improved vaccines in the 21st century. *Intervirology*, *45*(4-6), 199-211.
2. Yusibov, V., & Rabindran, S. (2008). Recent progress in the development of plant derived vaccines. *Expert review of vaccines*, *7*(8), 1173-1183.

3. Sohrab, S. S., Suhail, M., Kamal, M. A., Husen, A., & Azhar, E. I. (2017). Edible vaccine: current status and future perspectives. *Curr Drug Metab. https://doi. org/10.2174/1389200218666170711121810*.
4. Kim, T. G., & Yang, M. S. (2010). Current trends in edible vaccine development using transgenic plants. *Biotechnology and Bioprocess Engineering*, *15*, 61-65.
5. Fischer, R., Stoger, E., Schillberg, S., Christou, P., & Twyman, R. M. (2004). Plant-based production of biopharmaceuticals. *Current opinion in plant biology*, *7*(2), 152-158.
6. Rybicki, E. P. (2009). Plant-produced vaccines: promise and reality. *Drug discovery today*, *14*(1-2), 16-24.
7. Shah, C. P., Trivedi, M. N., Vachhani, U. D., & Joshi, V. J. (2011). Edible vaccine: A better way for immunization. *International journal of current pharmaceutical research*, *3*(1), 1-4.
8. Mor, T. S., Gómez-Lim, M. A., & Palmer, K. E. (1998). Perspective: edible vaccines—a concept coming of age. *Trends in microbiology*, *6*(11), 449-453.
9. Daniell, H., Streatfield, S. J., & Wycoff, K. (2001). Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends in plant science*, *6*(5), 219-226.
10. Hafiz, E., & Eyob, H. (2015). Review on edible vaccine. *Acad J Nutr*, *4*, 40-9.
11. Lal, P., Ramachandran, V. G., Goyal, R., & Sharma, R. (2007). Edible vaccines: current status and future. *Indian journal of medical microbiology*, *25*(2), 93-102.
12. Kurup, V. M., & Thomas, J. (2020). Edible vaccines: promises and challenges. *Molecular biotechnology*, *62*(2), 79-90.
13. Hirlekar, R., & Bhairy, S. (2017). Edible vaccines: An advancement in oral immunization. *Asian J. Pharm. Clin. Res*, *10*(78-84).
14. Esmael, H., & Hirpa, E. (2015). Review on edible vaccine. *Academic Journal of Nutrition*, *4*(1), 40-49.
15. Polshettiwar Satish, A., Swarupa, K., Gautam, V., Purva, C., & Aarti, S. (2023). A systematic review on edible vaccines. *Research Journal of Biotechnology Vol*, *18*, 1.
16. Webster, D. E., Thomas, M. C., Strugnell, R. A., Dry, I. B., & Wesselingh, S. L. (2002). Appetising solutions: an edible vaccine for measles. *Medical journal of Australia*, *176*(9), 434-437.

17. Richter, L., & Kipp, P. B. (1999). Topics in microbiology and immunology. *Plant Biotechnology: New Products and Applications. ed. Hammond J, McGarvey P, Yusibov V (Springer-Verlag, Heidelberg)*, 159-176.
18. Langridge WH. Edible vaccines. Sci Am. 2000; 283: 66-71
19. Naeema Jan[1], Fouzia Sha, et al, An Overview on Edible Vaccines and Immunization, Austin Journal of Nutrition and Food Sciences.
20. Sahoo, A., Mandal, A. K., Dwivedi, K., & Kumar, V. (2020). A cross talk between the immunization and edible vaccine: Current challenges and future prospects. *Life Sciences*, *261*, 118343.
21. Kurup, V. M., & Thomas, J. (2020). Edible vaccines: promises and challenges. *Molecular biotechnology*, *62*(2), 79-90.
22. Potent, A. B. V. P. (2004). Single Mucosal, but Not Parenteral. *J Immunol*, *173*, 6357-6365.
23. Lycke, N., & Bemark, M. (2010). Mucosal adjuvants and long-term memory development with special focus on CTA1-DD and other ADP-ribosylating toxins. *Mucosal immunology*, *3*(6), 556-566.
24. Lycke, N. (2012). Recent progress in mucosal vaccine development: potential and limitations. *Nature reviews immunology*, *12*(8), 592-605.
25. Hafiz, E., & Eyob, H. (2015). Review on edible vaccine. *Acad J Nutr*, *4*, 40-9.
26. Goldblatt, D., & Ramsay, M. (2003). Immunization in domestic animal. *Oxford text book of medicine fourth edition. Oxford University Press*, *32*(4), 378-396.
27. Levine MM. Enteric infection and the vaccines to counter them: Future directions . Natl Med 2006; 24(18): 3865-73.
28. Thomas, D. R., Penney, C. A., Majumder, A., & Walmsley, A. M. (2011). Evolution of plant-made pharmaceuticals. *International Journal of Molecular Sciences*, *12*(5), 3220-3236.
29. D'Aoust, M. A., Lavoie, P. O., Couture, M. M. J., Trépanier, S., Guay, J. M., Dargis, M., ... & Vézina, L. P. (2008). Influenza virus-like particles produced by transient expression in Nicotiana benthamiana induce a protective immune response against a lethal viral challenge in mice. *Plant biotechnology journal*, *6*(9), 930-940.
30. D'Aoust, M. A., Couture, M. M. J., Charland, N., Trépanier, S., Landry, N., Ors, F., & Vézina, L. P. (2010). The production of hemagglutinin-based virus-like particles in

plants: a rapid, efficient and safe response to pandemic influenza. *Plant biotechnology journal*, *8*(5), 607-619.

31. Pandupuspitasari, N. S. (2014). Recent developments in therapeutic protein expression technologies in plants. *Recent developments in therapeutic protein expression technologies in plants*.
32. Streatfield, S. J. (2007). Approaches to achieve high-level heterologous protein production in plants.
33. Thanavala, Y., Mahoney, M., Pal, S., Scott, A., Richter, L., Natarajan, N., ... & Mason, H. S. (2005). Immunogenicity in humans of an edible vaccine for hepatitis B. *Proceedings of the National Academy of Sciences*, *102*(9), 3378-3382.
34. Santi, L., Giritch, A., Roy, C. J., Marillonnet, S., Klimyuk, V., Gleba, Y., ... & Mason, H. S. (2006). Protection conferred by recombinant Yersinia pestis antigens produced by a rapid and highly scalable plant expression system. *Proceedings of the National Academy of Sciences*, *103*(4), 861-866.
35. Zhang, X., Buehner, N. A., Hutson, A. M., Estes, M. K., & Mason, H. S. (2006). Tomato is a highly effective vehicle for expression and oral immunization with Norwalk virus capsid protein. *Plant biotechnology journal*, *4*(4), 419-432.
36. Li, J. T., Fei, L., Mou, Z. R., Wei, J., Tang, Y., He, H. Y., ... & Wu, Y. Z. (2006). Immunogenicity of a plant-derived edible rotavirus subunit vaccine transformed over fifty generations. *Virology*, *356*(1-2), 171-178.
37. Guerrero-Andrade, O., Loza-Rubio, E., Olivera-Flores, T., Fehérvári-Bone, T., & Gómez-Lim, M. A. (2006). Expression of the Newcastle disease virus fusion protein in transgenic maize and immunological studies. *Transgenic research*, *15*, 455-463.
38. Moravec, T., Schmidt, M. A., Herman, E. M., & Woodford-Thomas, T. (2007). Production of Escherichia coli heat labile toxin (LT) B subunit in soybean seed and analysis of its immunogenicity as an oral vaccine. *Vaccine*, *25*(9), 1647-1657.
39. Jiang, X. L., He, Z. M., Peng, Z. Q., Qi, Y., Chen, Q., & Yu, S. Y. (2007). Cholera toxin B protein in transgenic tomato fruit induces systemic immune response in mice. *Transgenic research*, *16*, 169-175.
40. Rosales-Mendoza, S., Soria-Guerra, R. E., López-Revilla, R., Moreno-Fierros, L., & Alpuche-Solís, Á. G. (2008). Ingestion of transgenic carrots expressing the Escherichia coli heat-labile enterotoxin B subunit protects mice against cholera toxin challenge. *Plant cell reports*, *27*, 79-84.

41. Nochi, T., Takagi, H., Yuki, Y., Yang, L., Masumura, T., Mejima, M., ... & Kiyono, H. (2007). Rice-based mucosal vaccine as a global strategy for cold-chain-and needle-free vaccination. *Proceedings of the National Academy of Sciences*, *104*(26), 10986-10991.
42. Lugade, A. A., Kalathil, S., Heald, J. L., & Thanavala, Y. (2010). Transgenic plant-based oral vaccines. *Immunological investigations*, *39*(4-5), 468-482.
43. Joung, Y. H., Park, S. H., Moon, K. B., Jeon, J. H., Cho, H. S., & Kim, H. S. (2016). The last ten years of advancements in plant-derived recombinant vaccines against hepatitis B. *International Journal of Molecular Sciences*, *17*(10), 1715.
44. Das, S., & Deshmukh, R. (2009). Advances in vaccination: A review. *Int J Appl Pharm*, *1*, 1-21.
45. Singh BD. Biotechnology. 1st ed. India: Kalyani Publishers; 1998.
46. Hirlekar, R., & Bhairy, S. (2017). Edible vaccines: An advancement in oral immunization. *Asian J. Pharm. Clin. Res*, *10*(78-84).
47. Sohrab, S. S. (2020). An edible vaccine development for coronavirus disease 2019: the concept. *Clinical and experimental vaccine research*, *9*(2), 164.
48. Sartaj Sohrab, S., Suhail, M., A Kamal, M., Husen, A., & I Azhar, E. (2017). Recent development and future prospects of plant-based vaccines. *Current drug metabolism*, *18*(9), 831-841.
49. Jan, N., Shafi, F., Hameed, O. B., Muzaffar, K., Dar, S., Majid, I., & Nayik, G. A. (2016). An overview on edible vaccines and immunization. *Austin J Nutri Food Sci*, *4*(2), 1078.
50. Saxena, J., & Rawat, S. (2014). Edible vaccines. *Advances in biotechnology*, 207-226.
51. Govea-Alonso, D. O., Rybicki, E., & Rosales-Mendoza, S. (2014). Plant-based vaccines as a global vaccination approach: Current perspectives. *Genetically Engineered Plants as a Source of Vaccines Against Wide Spread Diseases: An Integrated View*, 265-280.
52. Daniell, H., Streatfield, S. J., & Wycoff, K. (2001). Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends in plant science*, *6*(5), 219-226.
53. Hiatt, A., Caffferkey, R., & Bowdish, K. (1989). Production of antibodies in transgenic plants. *Nature*, *342*(6245), 76-78.
54. Sahoo, A., Mandal, A. K., Dwivedi, K., & Kumar, V. (2020). A cross talk between the immunization and edible vaccine: Current challenges and future prospects. *Life Sciences*, *261*, 118343.

55. Mishra, N., Gupta, P. N., Khatri, K., Goyal, A. K., & Vyas, S. P. (2008). Edible vaccines: A new approach to oral immunization.
56. Chen, Q., & Lai, H. (2015). Gene delivery into plant cells for recombinant protein production. *BioMed research international*, *2015*(1), 932161.
57. Gomez, E. R., Chimeno Zoth, S. A., Carrillo, E. C., & Berinstein, A. (2010). *Developments in plant-based vaccines against diseases of concern in developing countries*. BENTHAM Open.
58. Shah, C. P., Trivedi, M. N., Vachhani, U. D., & Joshi, V. J. (2011). Edible vaccine: A better way for immunization. *International journal of current pharmaceutical research*, *3*(1), 1-4.
59. Kurup, V. M., & Thomas, J. (2020). Edible vaccines: promises and challenges. *Molecular biotechnology*, *62*(2), 79-90.
60. de la Riva, G. A., González-Cabrera, J., Vázquez-Padrón, R., & Ayra-Pardo, C. (1998). Agrobacterium tumefaciens: a natural tool for plant transformation. *Electronic journal of Biotechnology*, *1*(3), 24-25.
61. Aswathi, P. B., Bhanja, S. K., Yadav, A. S., Rekha, V., John, J. K., Gopinath, D., ... & Jacob, A. (2014). Plant based edible vaccines against poultry diseases: A review. *Adv. Anim. Vet. Sci*, *2*(5), 305-311.
62. Hafiz, E., & Eyob, H. (2015). Review on edible vaccine. *Acad J Nutr*, *4*, 40-9.
63. Hirlekar, R., & Bhairy, S. (2017). Edible vaccines: An advancement in oral immunization. *Asian J. Pharm. Clin. Res*, *10*(78-84).
64. Shah, C. P., Trivedi, M. N., Vachhani, U. D., & Joshi, V. J. (2011). Edible vaccine: A better way for immunization. *International journal of current pharmaceutical research*, *3*(1), 1-4.
65. Lal, P., Ramachandran, V. G., Goyal, R., & Sharma, R. (2007). Edible vaccines: current status and future. *Indian journal of medical microbiology*, *25*(2), 93-102.
66. Thanavala, Y., Mahoney, M., Pal, S., Scott, A., Richter, L., Natarajan, N., ... & Mason, H. S. (2005). Immunogenicity in humans of an edible vaccine for hepatitis B. *Proceedings of the National Academy of Sciences*, *102*(9), 3378-3382.
67. Concha, C., Cañas, R., Macuer, J., Torres, M. J., Herrada, A. A., Jamett, F., & Ibáñez, C. (2017). Disease prevention: an opportunity to expand edible plant-based vaccines?. *Vaccines*, *5*(2), 14.

68. Srinivas, L., Sunil Kumar, G. B., Ganapathi, T. R., Revathi, C. J., & Bapat, V. A. (2008). Transient and stable expression of hepatitis B surface antigen in tomato (Lycopersicon esculentum L.). *Plant biotechnology reports*, *2*, 1-6.
69. Tacket, C. O., & Mason, H. S. (1999). A review of oral vaccination with transgenic vegetables. *Microbes and infection*, *1*(10), 777-783.
70. Mor, T. S., Gómez-Lim, M. A., & Palmer, K. E. (1998). Perspective: edible vaccines—a concept coming of age. *Trends in microbiology*, *6*(11), 449-453.
71. Oszvald, M., Kang, T. J., Tomoskozi, S., Tamas, C., Tamas, L., Kim, T. G., & Yang, M. S. (2007). Expression of a synthetic neutralizing epitope of porcine epidemic diarrhea virus fused with synthetic B subunit of Escherichia coli heat labile enterotoxin in rice endosperm. *Molecular biotechnology*, *35*, 215-224.
72. Qian, B., Shen, H., Liang, W., Guo, X., Zhang, C., Wang, Y., ... & Zhang, D. (2008). Immunogenicity of recombinant hepatitis B virus surface antigen fused with preS1 epitopes expressed in rice seeds. *Transgenic Research*, *17*, 621-631.
73. Karasev, A. V., Foulke, S., Wellens, C., Rich, A., Shon, K. J., Zwierzynski, I., ... & Reitz, M. (2005). Plant based HIV-1 vaccine candidate: Tat protein produced in spinach. *Vaccine*, *23*(15), 1875-1880.
74. Ye YanJu, Y. Y., & Li WenGui, L. W. (2010). Immunoprotection of transgenic alfalfa (Medicago sativa) containing Eg95-EgA31 fusion gene of Echinococcus granulosus against eg protoscoleces.
75. Zhang, H., Liu, M., Li, Y., Zhao, Y., He, H., Yang, G., & Zheng, C. (2010). Oral immunogenicity and protective efficacy in mice of a carrot-derived vaccine candidate expressing UreB subunit against Helicobacter pylori. *Protein Expression and Purification*, *69*(2), 127-131.
76. Kumar, G. S., Ganapathi, T. R., Revathi, C. J., Srinivas, L., & Bapat, V. A. (2005). Expression of hepatitis B surface antigen in transgenic banana plants. *Planta*, *222*, 484-493.
77. Arakawa, T., Chong, D., & Langridge, W. (1998). Transgenic plants for the production of edible vaccine and antibodies for immunotherapy. *Nature Biotechnology*, *16*, 292-297.
78. Patel, P., Patel, R., Patel, S., Patel, Y., Patel, M., & Trivedi, R. (2022). Edible Vaccines: A Nutritional Substitute for Traditional Immunization. *Pharmacognosy Reviews*, *16*(32).

79. Richter, L. J., Thanavala, Y., Arntzen, C. J., & Mason, H. S. (2000). Production of hepatitis B surface antigen in transgenic plants for oral immunization. *Nature biotechnology*, *18*(11), 1167-1171.
80. Lou, X. M., Yao, Q. H., Zhang, Z., Peng, R. H., Xiong, A. S., & Wang, H. K. (2007). Expression of the human hepatitis B virus large surface antigen gene in transgenic tomato plants. *Clinical and Vaccine Immunology*, *14*(4), 464-469.
81. Marcondes, J., & Hansen, E. (2008). Transgenic lettuce seedlings carrying hepatitis B virus antigen HBsAg. *Brazilian Journal of Infectious Diseases*, *12*, 469-471.
82. William, H., 2000. Edible Vaccines by using potato. Altenatives to injectable vaccine.Scientific American, 12(1): 67.
83. Tacket, C. O., Mason, H. S., Losonsky, G., Clements, J. D., Levine, M. M., & Arntzen, C. J. (1998). Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. *Nature medicine*, *4*(5), 607-609.
84. Prakash, C. S. (1996). Edible vaccines and antibody producing plants.
85. Karasev, A. V., Foulke, S., Wellens, C., Rich, A., Shon, K. J., Zwierzynski, I., ... & Reitz, M. (2005). Plant based HIV-1 vaccine candidate: Tat protein produced in spinach. *Vaccine*, *23*(15), 1875-1880.
86. Koya, V., Moayeri, M., Leppla, S. H., & Daniell, H. (2005). Plant-based vaccine: mice immunized with chloroplast-derived anthrax protective antigen survive anthrax lethal toxin challenge. *Infection and immunity*, *73*(12), 8266-8274.
87. Lebens, M., Johansson, S., Osek, J., Lindblad, M., & Holmgren, J. (1993). Large-scale production of Vibrio cholerae toxin B subunit for use in oral vaccines. *Bio/Technology*, *11*(12), 1574-1578.
88. Yuki, Y., & Kiyono, H. (2003). New generation of mucosal adjuvants for the induction of protective immunity. *Reviews in medical virology*, *13*(5), 293-310.
89. Polshettiwar Satish, A., Swarupa, K., Gautam, V., Purva, C., & Aarti, S. (2023). A systematic review on edible vaccines. *Research Journal of Biotechnology Vol*, *18*, 1.
90. Sala F, Manuela Rigano M, Barbante A, Basso B, Walmsley AM, Castiglione S. Vaccine antigen production in transgenic plants: Strategies, gene constructs and perspectives. Vaccine. 2003;21(7-8):803-8. doi: 10.1016/s0264- 410x(02)00603-5, PMID 12531364.