ISSN: 3107-6513

Review on Role of Edible Vaccines for Oral Immunization

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Abstract:

Oral vaccines are gaining more attention due to their ease of administration, lower invasiveness, generally greater safety, and lower cost than injectable vaccines. This review introduces certified oral vaccines for adenovirus, recombinant protein-based, and transgenic plant-based oral vaccines, and their mechanisms for inducing an immune response. Procedures for regulatory approval and clinical trials of injectable and oral vaccines are also covered. Challenges such as instability and reduced efficacy in low-income countries associated with oral vaccines are discussed, as well as recent developments, such as Bacillus-subtilis-based and nanoparticle-based delivery systems that have the potential to improve the effectiveness of oral vaccines. The scientific evidences enlighten that plants are the rich source of oral vaccines, which can be given either by eating the edible parts of plants and/or by oral administration of highly refined proteins. The use of plant-based edible vaccines is an emerging trend as it possesses minimum or no side effects compared with synthetic vaccines. This review article gives insights into different types of vaccines, the use of edible vaccines, advantages of edible vaccines over conventional vaccines, and mechanism of action of edible vaccines.

Keywords: Oral vaccine,gene therapy, immunity,conventional drugs.

1. Introduction

The development of vaccines was one of the most important breakthroughs in healthcare and medicine. Preceding Edward Jenner's creation of the smallpox vaccine infectious diseases had killed countless people. Since then, vaccines have had a profound impact, saving millions of lives by eradicating the spread of devastating diseases such as polio,measles, and diphtheria. As a result, vaccines have been considered among the most effective public health interventions.

Despite being efficient at preventing diseases, conventional vaccine delivery techniques using injections through intramuscular and intravenous routes also come with a variety of drawbacks, for example, requiring qualified medical staff, storage of vaccines at low temperatures, and pain at the injection site.Researchers have focused on the creation of alternate, less invasive vaccine delivery systems, such as oral vaccinations, to overcome these issues.[1]

A milestone in vaccine history occurred with the introduction of the first oral vaccinations in the 1960s; the oral polio vaccine (OPV) was applied in the fight to eradicate polio.After the success of OPV, numerous oral vaccines were developed and proven to be effective in preventing diseases including cholera, rotavirus, and typhoid.With administration into the gut directly, the primary target of oral vaccines would be the gut-associated lymphoid tissue (GALT), a crucial region for vaccination-induced immune responses. The ease of administration and simple manufacturing procedures are also advantages of oral vaccine administration over conventional injection-type vaccines.Moreover, oral vaccinations are a desirable alternative for individuals with weakened immune systems,

ISSN: 3107-6513

because the blood vessels and circulatory system are avoided.

What is vaccines ?

A vaccine is a biological preparation that helps the body develop immunity to a disease. It typically contains a weakened or inactive form of a disease-causing microorganism (like a virus or bacteria) or a part of it. This agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it. This immunological memory allows the body to more effectively fight off the actual disease if encountered later.

What are edible vaccines ?

An edible vaccine is a type of vaccine created by genetically modifying edible plant parts to express antigens. When consumed, these antigens stimulate an immune response in the body, offering a potentially cost-effective and easily administered alternative to traditional vaccines.[2]

Vaccination

Vaccination is the practice of delivering antigens to the body in order to induce an immune response. To begin with, during an infection there is a particular time lapse during the onset of an infection and the production of antibodies. The body‘s immune cells take some time to identify the epitope of the pathogen and produce specific antibodies. This time lapse can be life-threatening for patients suffering from infections of highly-potent pathogens. However, the antibody titers are usually very low. This is known as the primary response. A vaccine usually confers antibody production at faster rate and at higher levels. This is called the secondary response. So the infection is rapidly subsided. Vaccines also produce immunogenic memory- memory cells record the configuration of the antigen and remember them for future infections. Vaccination is very important for the individual as well as the country. By getting vaccinated, the individual is being protected from serious and life-threatening diseases that could occur in the future.

2. History and background

Many people in developing countries do not have access to the vaccines they need, as the traditional vaccines are costly and require skilled medical people for administration and are less effective in inducing mucosal immune response. It was these needs which inspired who attempted to produce antibodies in plants which could serve the purpose of passive immunization. The first report of edible vaccine (a surface protein from streptococcus) in tobacco, at 0.02 % of total leaf protein level, appeared in 1990 in the form of a patent application published under the international patent cooperation treaty. By conceiving the idea of edible vaccine dr. Charles arntzen tried to realize.[3]

In 1992, arntzen and coworkers introduced the concept of transgenic plants as a production and delivery system for subunit vaccines in which edible tissues of transgenic crop plants were used They found that this concept could overcome the limitations of traditional vaccines, thereby triggering the research on edible vaccine. In 1990s, streptococcus mutans surface protein antigen a was expressed for the first time in tobacco. The same group also pioneered the field with work on hepatitis b and heat-labile toxin, b subunit in tobacco plants and potato tubers. In the same year, the successful expression of hepatitis b surface antigen in tobacco plants was also achieved. To prove that plant-derived hbsag could stimulate mucosal immune responses via oral route, potato tubers were used as an expression system and were optimized to increase the accumulation of the protein in plant tubers parallel to the evaluation of plant-derived hbsag, mason and arntzen explored plant expression of other vaccine candidates including the labile toxin b subunit of enterotoxigenic escherichia coli and the capsid protein of norwalk virus.

ISSN: 3107-6513

The plant-derived proteins correctly assembled into functional oligomers that could elicit the expected immune responses when given orally to animals in 1998 a new era was opened in vaccine delivery when researchers supported by the national institute of allergy and infectious diseases have shown for the first time that an edible vaccine can safely generate significant immune responses in people. The report by collaborators from the university of maryland in baltimore, the boyce thompson institute for plant research in ithaca, n.y., and tulane university in new orleans appeared in the may issue of nature medicine. According to the then director of NIAID "edible vaccines offer exciting possibilities for significantly reducing the burden of diseases like hepatitis and diarrhea, particularly in the developing world where storing and administering vaccines are often major problems," also discussed the rapid increase of research in the edible-vaccine field and pointed out that plants could be used to create multicomponent vaccines that can protect against several pathogens at once. This is an aspect of the edible-vaccine approach that further strengthens its impact. Later, in 2003 sala and research group reported that proteins produced in these plants induced the mucosal immune response which was the main aim behind this concept.research into edible vaccine is still at a very early stage and scientists have a long way to go before it will become a major part of immunization program world wide.

In 1990, a significant advancement in edible vaccine production was witnessed by successfully detecting antibody response in mice with Streptococcus surface protein antigen expressed in tobacco plants 1998. The initial human trial in 1998 used engineered potatoes with a modified fragment of the diarrhea-causing Escherichia coli toxin and demonstrated a four-fold increase in intestinal antibodies after immunization Transgenic potatoes had no significant side effects, indicating the first successful induction of a human immune response through an edible vaccine In 1996, the expression of hepatitis B surface antigen (HBsAg) in potato tubers revealed that plant-derived HBsAg may stimulate mucosal immune responses upon oral consumption demonstrated that proteins synthesized by these plants could stimulate immune responses in mucosal tissues[4]The concept of utilizing transgenic plant edible organs (fruits and tubers) to deliver subunit vaccines emerged as a promising approach to overcoming the challenges associated with conventional vaccines. Subsequent investigations effectively expressed the hepatitis B and heat-labile toxin β subunits in tobacco plants and potato tubers The HBsAg for Hepatitis B was successful in several edible crops, including cherry tomato banana tomato rice and algae Similarly, the list of plants for edible vaccines for other diseases was discussed in the next sections.Despite being in its nascent phase, edible vaccination research has established an initial foundation that provides a framework for future progress. There remains a considerable distance to traverse before edible vaccinations are widely used in vaccination programs worldwide.

3. Production of edible vaccines

Antigens that are delivered into the body are divided in to two categories : Proteins and Peptides. The antigen is either the full length protein or a peptide fragment of protein. The decision to utilize a protein or peptide antigen is case specific and is influenced by a variety of circumstances. Both plant viruses were utilized to establish the two major techniques for expressing the immunogenic protein or peptide in the host plant .Epitope presentataion systems and polypeptide expression systems are the first and second,respectively short antigenic peptides fused to the coat protein(CP)that are presented on the surface of formed viral particles are employed in epitope presentation systems. The complete unfused recombinant protein that accumulates with in the plants is expressed by polypeptide expression systems. Edible vaccines are subunit vaccines : they contain proteins for a pathogen to

ISSN: 3107-6513

form .The first steps in making an edible vaccine is the identification , isolation and characterization of a pathogenic antigen.In order to be effective the antigen needs to elicit a strong and specific immune response. Once the antigen is identified and isolated , the gene is cloned into a transfer vector . one of the most common transfer vectors of DNA being used for edible vaccines is agrobacterium tumefaciens . The pathogens sequence is inserted in to the transfer DNA to produce the antigenic protein .

4. Mechanism of action

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[19]. 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).[5] IgA then forms the secretory IgA, which is then transported into the lumen. Production of secretory. Immune suppression by using triamcinolone. However, this has to be done in small amounts so as prevent any major health concerns or even. Increasing the dosage of the vaccine significantly can often lead to jump starting the immune response.

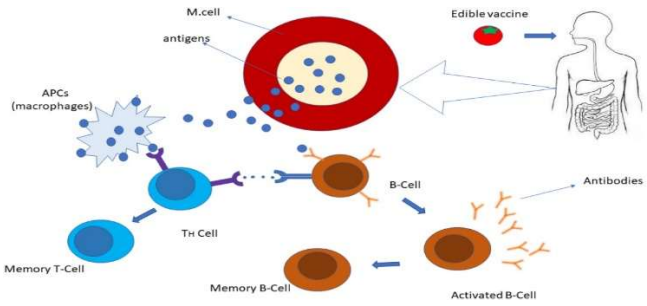

Fig:1. Mechanism of action

5. Edible vaccines from the plant source

Many plants have been identified and studied for the edible vaccine which was transformed to express antigen for rotavirus, gastroenteritis, cholera, autoimmune diseases and rabies. Moreover, several experiments have used vegetable potato, but potatoes may not be the ideal choice for edible vaccines since frying or boiling will degrade certain antigenic proteins. Certain foods, such as bananas, tomato, carrots peanuts corn and tobacco have a more promising potential as edible vaccines as it can be eaten raw, not only because they are commonly available, but since genetic engineering is efficiently developed these kinds of vegetable plants.

ISSN: 3107-6513

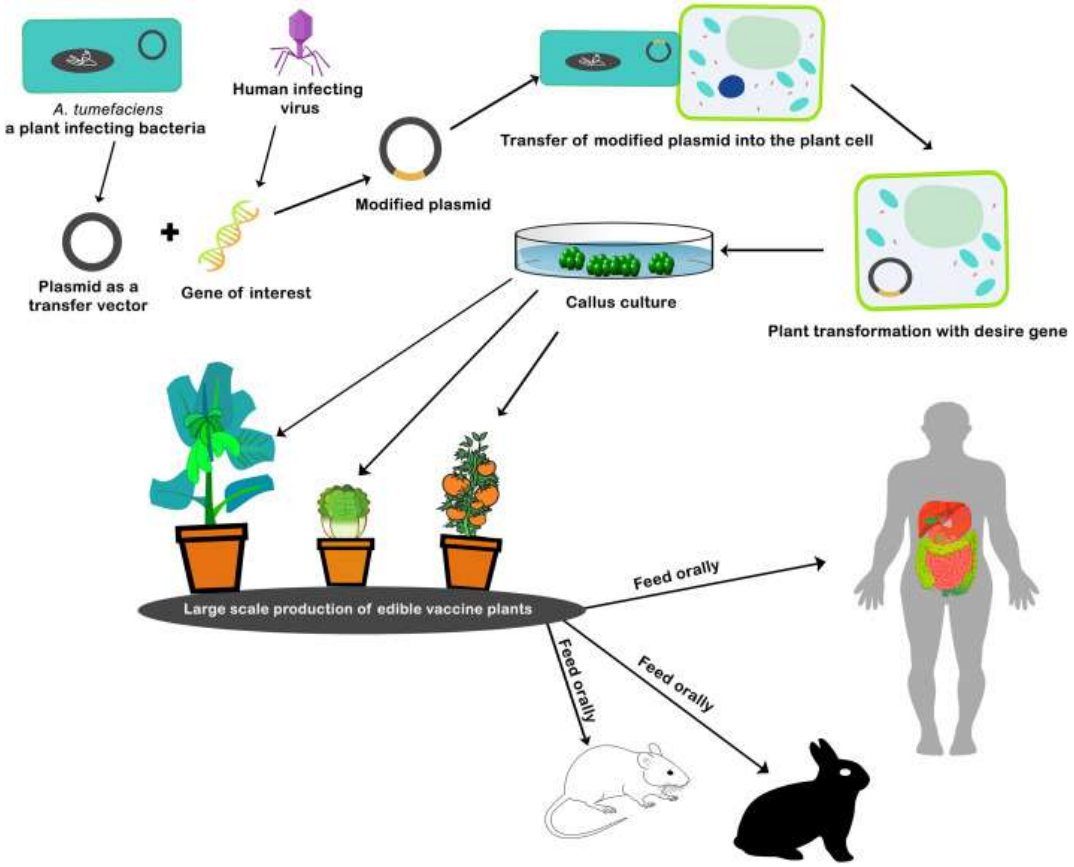

Fig:2 Procedure involved in development of edible vaccines

- Potato

The effectiveness of the antigens produced from potatoes (*Solanum tuberosum*) toward the non-toxic subunit of Vibrio cholerae endotoxin and the Norwalk virus capsid pathogen was identified in rats and human volunteers.

Fig:3. potato

- Tomato/Tobacco

The genome of tomato and tobacco is incorporated with N-terminal fragment of SARS-CoV protein (S1) used to develop the safe, effective and inexpensive vaccine. When these plant-based vaccines for SARS give to mice, shows significant increase level of SARS-CoV-specific IgA after oral ingestion of tomato, expressing S1 protein. Whereas tobacco-derived S1-protein indicate the presence of SARS-CoV-specific IgG detect by ELISA analysis and Western blot.Tobacco is not an edible plant but play a major role in the development of the vaccine as it is used as a proof-of-concept model species for the edible vaccine.[6]

Fig:4. Tomato &tobacco

ISSN: **3107-6513**

- Cherry tomatallis

For HBsAg gene of hepatitis B, lines of transgenic cherry tomatillos have been grown. The expression of genes was seen through the whole plant, but it was maximum in the fresh leaves weight of 300 ng/g and with fresh fruit weight of 10 ng/g .

- Lettuce

Lactuca *sativa* express the B-subunit of the thermolabile protein of *E. coli*, responsible for both human and animal enteric disease, show the possibility of this vegetable as an edible vaccine. In 2005, the typical swine fear hog pest virus glycoprotein E2 was expressed by lettuce. In Poland, the transgenic lettuce that shows effect against hepatitis B virus is in the development stage.

Fig:5. lettuce

- Soya bean

E. coli bacteria B-subunit of thermolabile toxin, expression was performed in the endoplasmic reticulum (ER) of soybean (*Glycine max*), which yielded a total antigen level of up to 2.4% of the total soybean seed protein without any problem during drying for further processing.[7]

Fig:6. Soya bean

- Algae

Chlamydomonas reinhardtii (green algae) has been used as a tool to achieve a large number of proteins specific to both animal and humans for therapeutic purpose. The use of algae for the production of vaccines is optimistic, as algae have a very high growth rate, the entire system can be used as a raw material for the development of edible vaccines.

- Pea

Based on the expression of the capsid protein Norwalk virus, the transgenic plant was developed. Protein deposition in the un-ripened fruit with a lower accumulation in red fruit was reported up to 8% of soluble protein. Expression in seeds allowed the storage of antigenic peptides, thus creating a plant with a high yield of proteins, with an average protein content of about 20%–40%, which would preclude intensive purification procedure by pharmaceutical industries.[8]

- Banana

carrot along with *A. thaliana* was utilized to develop an edible vaccine for surface HIV antigen expression, and in the study, it was reported that rats showed more positive effect compared to those non-treated animals. Carrot (*Daucus carota*) has a positive effect in the treatment of HIV not only because carrots are nutritious and tasty, but because of carrot main chemical constituent carotenoids which on consumption by rats increases monocytes, lymphocytes, and other immune defence. Thus, people with a weakened immune system might benefit from the use of this potential edible anti-HIV vaccine.

ISSN: 3107-6513

Fig:7. banana

- Rice

A research in 2007 found that transgenic rice (*Oryza sativa*) plants expressing the B subunit of *E. coli* induces significant number of antibodies to this subunit. In the same year, an immune response was found to be caused in chicken by transgenic rice that is a result of the VP2 antigenic protein from infectious bursitis.[9]

Fig:8. Rice (oryza sativa)

6. Principles of edible vaccines

The development of edible vaccines is based on the principles of genetic engineering, plant biotechnology, and immunology These vaccines rely on the ability of plants to produce antigenic proteins that, when consumed, elicit an immune response in the host.The key principles governing edible vaccines include:

a. Genetic Modification of Plants:

The first step in edible vaccine production involves identifying and isolating the gene encoding a specific antigen from a pathogen. This gene is then introduced into the genome of an edible plant using genetic engineering techniques such as Agrobacterium-mediated transformation, biolistic methods or electroporation. The transgenic plant subsequently expresses the antigenic protein in its edible tissues.[10]

b. Expression of Antigenic Proteins:

Once integrated into the plant genome, the antigen-encoding gene is transcribed and translated into functional proteins. The selection of appropriate promoters and enhancers ensures optimal levels of antigen expression. Chloroplast transformation, an advanced technique, enhances antigen yield and stability by inserting genes into the chloroplast genome rather than the nuclear genome

c. Oral Administration and Immunogenicity:

Upon consumption, the plant-derived antigenic proteins pass through the digestive tract. In the intestine, the antigen is recognized by mucosal-associated lymphoid tissues (MALT), such as Peyer's patches, where it stimulates an immune response[11].The activation of mucosal immunity is crucial in preventing infections at their primary entry sites

d. Induction of Systemic and Mucosal Immunity:

Edible vaccines stimulate both humoral (antibody-mediated) and cell-mediated immune responses. The production of secretory IgA in mucosal surfaces helps prevent pathogen colonization, while systemic IgG antibodies provide long-term immunity Additionally, antigen-presenting cells (APCs) such as dendritic cells and macrophages play a role in processing and presenting the antigen to T cells, thereby activating adaptive immunity.

e. Plant Selection and Optimization:

Various plant species have been explored for edible vaccine production, including potatoes, tomatoes, bananas, rice, lettuce, and carrots.The choice of plant depends on factors such as antigen expression efficiency, ease of cultivation, and suitability for raw consumption. Non-allergenic

ISSN: 3107-6513

and heat-stable plants are preferred to maintain vaccine efficacy.

Unlike conventional vaccines that require cold-chain logistics, edible vaccines offer the advantage of stability under ambient conditions. However, ensuring long-term stability post-harvest remains a challenge, necessitating strategies such as freeze-drying or encapsulation to protect antigen integrity during storage and transportation [12]

f. Safety and Regulatory Considerations:

While edible vaccines hold great promise, their development is subject to stringent safety and regulatory assessments. Concerns such as potential allergenicity, dosage standardization, and environmental risks associated with genetically modified organisms (GMOs) must be addressed before commercialization. By leveraging these principles, edible vaccines offer an innovative, cost-effective, and scalable solution for immunization, particularly in regions where traditional vaccination programs face logistical and economic constraints. Ongoing research aims to optimize antigen expression, enhance immunogenicity, and establish regulatory frameworks to ensure the widespread adoption of edible vaccines in global healthcare initiatives. [13]

➢ *EDIBLE VACCINES USED FOR VARIOUS DISEASES*

Norwalk disease

Norwalk disease is caused by Norwalk virus, a member of the family . It causes acute gastroenteritis in human beings. Norwalk virus genome was cloned and that has facilitated the production of various vaccines. Norwalk virus capsid protein was expressed in insect cells. The resulting protein lacked the viral RNA thus making it non-pathogenic. The particles closely resembled an authentic Norwalk virus both antigenically and morphologically.Plant expression vectors pNV101, pNV102, and pNV140 were constructed by Mason .These plasmids were then transformed by using Agrobacterium tumifaciens LBA4404 by the freeze-thaw method. The Norwalk virus coat protein (NVCP) was then quantified with ELISA using rabbit anti (i-rNV) serum diluted 1:10000 in 0.01 M PBS. The recombinant Norwalk virus-like particles were extracted from plant tissue and then purified. This purified protein was then quantified and qualified using anion exchange chromatography, SDS PAGE, and western blotting. Mice were fed with the recombinant proteins and they showed production of humoral and mucosal antibody responses.[14]

Hepatitis b

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV), which affects the liver. It rarely leads to death. The hepatitis B surface antigen (HBsAg) was expressed in transgenic lupin callus by feeding the mice with transgenic lupin callus tissue and HBsAg specific antibodies. The DNA that encodes for the surface antigen of HBV was cloned. The plasmid carrying the HBsAg coding sequence was electroporated into Agrobacterium tumifaciens LBA4404 and C58. C58 was used for transforming seedlings of yellow lupin and LBA4404 was used on the lettuce plant. Both transformations were successful and the protein was extracted and analyzed by plotting a standard curve based on different concentrations of HBsAg. The transgenic lupin tissue was fed to mice and human volunteers were fed with transgenic lettuce. ELISA was carried out on both the mice and the human volunteers' serum samples. Both samples showed antibody titers.

Cholera

Cholera is a bacterial disease caused by Vibrio cholerae, a Gram-negative, comma-shaped bacteria that causes acute watery diarrhea by colonizing the small intestine and producing an enterotoxin, cholera toxin B. CTB acts as a potent mucosal immunogen when taken orally. This is the result of the CTB binding to the eukaryotic cell surfaces via the GM1 ganglioside receptors present on the epithelial surface of the intestines, thus eliciting a mucosal response to pathogens.

ISSN: 3107-6513

Immune response is enhanced when it is chemically coupled to other antigens. In an experiment carried out by Daniel. the construction of the chloroplast expression vector, pLD-LH-CTB, was carried out. The CTB production in E. coli was analyzed using immunblot assay. Then, the plasmid DNA were bombarded into the Nicotina tobacum leaves. The transformed leaves were cut and grown in a medium containing a selection marker, in this case streptomycin PCR analysis was done followed by southern blot analysis. Western blot analysis and ELISA were used to quantify the amount of CTB protein produced. Finally, GM1 ganglioside assay was done showing that both the chlorophyll-synthesized CTB and the bacterial CTB demonstrated a strong affinity for GM1 ganglioside. High levels of constitutive expression of CTB in transgenic tobacco do not affect the growth rate, flowering, and seeding, unlike when expressed in nuclear genome[15]

7. Advantages of edible vaccines

i. Edible vaccines are produced from inexpensive plants to cultivate, harvest, and process, which is more cost effective than traditional vaccines.
ii. It does not require specialized storage facilities and is easier to store and transport to remote areas.
iii. It can be administered orally, eliminating the need for needles and syringes to reduce the risk of infections and injury.[16]
iv. Further, the edible vaccine is administered with the help of needles, reducing the risk of contamination from the blood-borne pathogen. Also, the potential for simultaneous delivery of multiple vaccines.

Disadvanntages of edible vaccines

i. It may not be as effective as a traditional vaccine as the immune response generated is less robust than the vaccine administered via injection.
ii. It is difficult to control the vaccine dosage delivered to each individual, which can lead to inconsistencies in the immune response generated by the vaccine.
iii. The edible vaccine may face regulatory hurdles that traditional vaccines do not.[17]

8. Applications of edible vaccines

1. Target Diseases:

Infectious Diseases: Edible vaccines are being developed for a range of infectious diseases, including hepatitis B, cholera, measles, and HIV.

***Diarrheal Diseases*:** Vaccines for enterotoxigenic Escherichia coli (ETEC) and rotavirus are under investigation, targeting significant causes of childhood mortality in developing countries.

2. Plant Platforms:

***Common Plants*:** Potatoes, tomatoes, bananas, lettuce, and rice are among the plants explored for edible vaccine production.

***Research Focus*:** Research is focused on optimizing antigen expression, stability, and immune response efficacy.

3. Clinical Trials:

***Human Trials*:** Some edible vaccines have progressed to human clinical trials. For example, potatoes expressing LT-B antigen have shown promise in inducing an immune response against ETEC.[18]

Animal Studies: Extensive animal studies are being conducted to evaluate the safety and efficacy of various edible vaccine candidates.

9. Challenges and limitations

1.Dose Standardization:

***Variable Antigen Levels*:** Ensuring consistent levels of antigens in each plant or plant part is challenging, leading to difficulties in standardizing dosages.

ISSN: 3107-6513

***Dosage Control*:** Accurate dosing can be problematic due to natural variations in plant material.

2. *Regulatory Hurdles:*

***Approval Process*:** Regulatory approval for genetically modified organisms (GMOs) and edible vaccines is complex and varies between countries.

***Safety Concerns*:** Potential risks associated with GMOs need to be thoroughly assessed, including allergenicity and environmental impact.[19]

3. Public Acceptance:

***GMO Controversy*:** Public skepticism and resistance to genetically modified foods could hinder the acceptance of edible vaccines.

Education and Awareness: Extensive public education campaigns are needed to promote understanding and acceptance of edible vaccines.

4. Immune Tolerance:

***Oral Tolerance*:** Repeated oral exposure to antigens could lead to immune tolerance rather than an active immune response, reducing vaccine efficacy.[20]

Conclusion

Edible vaccines are pharmaceuticals derived from the expression of antigens in transgenic plants. It is a concept, which has been introduced in the 1990's and developed by Dr. Charles Arntzen. It is a convenient vaccine delivery system for developing countries since oral immunization has several advantages. The high costs, storage and transportation issues of conventional vaccines would vanish by using edible vaccines, however the dosage of the edible vaccine is difficult to control if you do not have any personal information like weight or ripeness/size of the fruit. The plant based edible vaccines are currently developed for a variety of humans and animal diseases.

A time period of 10-20 years is estimated until edible vaccines can be used as daily medical products. The future of the research depends on people being less afraid to use recombinant plants. This new concept of oral immunization could save millions of lives, especially in poorer countries, where vaccination against infectious diseases is needed. Edible vaccines are used as a protection against diseases rather than a cure manufacture encoded.

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