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# A Comprehensive Review of Approaches to Edible Vaccine

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**ABSTRACT:** Edible vaccine is a pharmaceutical derived from the expression of antigens in transgenic plants. It is a concept, which has been introduced in the 1990s and developed by Dr.charlesArntzen. This new concept of oral immunization could say millions of lives, especially in proper countries, where vaccination against infectious diseases in needed. Edible vaccines are used as a protection against diseases rather than a cure. Edible vaccines are cost effective, easy to administer and they are readily acceptable vaccine delivery systems, especially for poor developing countries. 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.

**KEYWORDS:**Edible vaccine, Recombinant plant, Immune system, Transgenic plant.

## I. INTRODUCTION:

More than one million people die each year of infectious diseases. 50% of these diseases are caused by pathogens infecting the mucosal membrane of the mammalian host.[1] The challenge today is to find unique and innovative vaccines that can target pathogens and infections at various stages. Vaccines are biological preparations that improve our immunity. The concept of vaccination was first put forth by Edward Jenner in 1796 for small pox. Vaccination is the process by which the body is made ready to face and fight off new infections. This way of treatment is indirect 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 immunizes 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 in accessible in developing countries.[2,3] 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.[4,5] Post translation modifications that generally occur in eukaryotic expression systems may positively effect the immunogenicity of the expressed antigen.[6] For a long time, mammalian recombinant expression systems were used to express such proteins even though mammalian systems are very difficult to handle and expensive. They also have low expression levels making them a bad choice to be used as a protein expression plateform. [7]

**MECHANISM OF ACTION:** Edible vaccines are required to induce the activation of the muscosal immune response system(MIS) The MIS is the first line of defence 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.[8] 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 Tcells ,whichb then activate B cells . Activeted B cells then migrate to the mesenteric lymph nodes were they mature in to plasma cells , which then migrate to mucosal membranes to secrete immunoglobulin A (IgA). IgA then forms the secretory IgA, which is then transported into 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-

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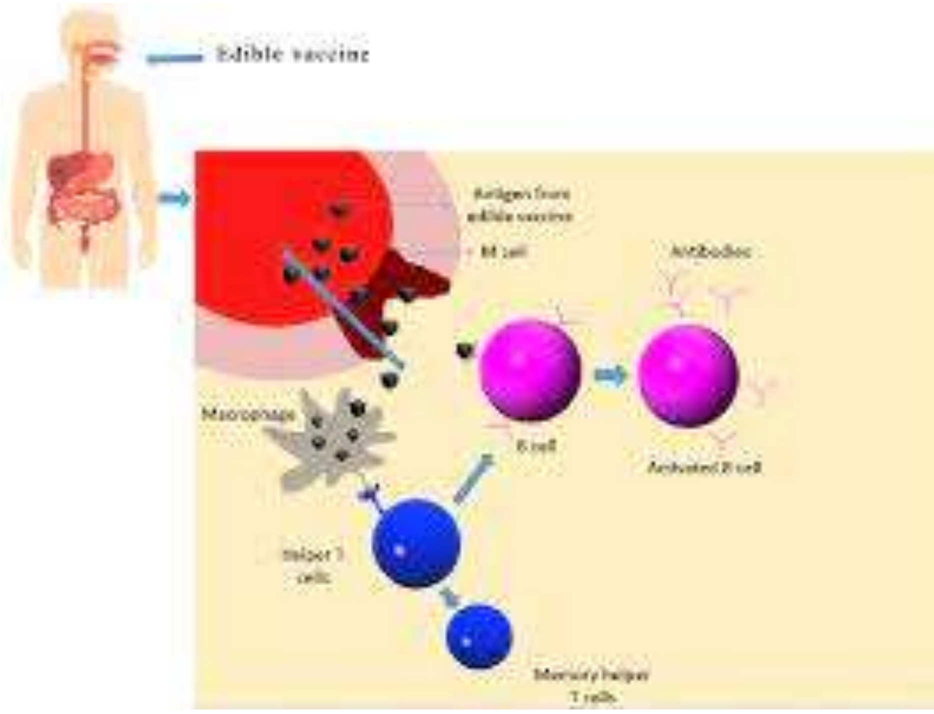
cell-independent fashion. These sIgA are poly reactive and usually recognise the foreign antigens .In the lumen, the sIgA neutralizes the invading pathogen by reacting with specific antigenic epitopes[9] as shown .The ,ost common problem most oral vaccines/therapeutics phase is the the tolerance towards the vaccine in the gut . This problem can be over come by some methods

- Immune suppression by using triamcinolone .how ever , this has to be done in small amounts so as prevent any major health concern or even fatality .
- Increasing the dosage of the vaccine significantly can often lead to jump starting the immune response.
- Multiple doses over a specific period of time as suggested by Silin and Lyubomska [10].




**CONCEPT OF EDIBLE VACCINE**:Edible vaccines are created by introducing the desired gene in to a plant to manufacture the encoated protein. The coat protein of a specific virus or bacteria that has no pathogenicity is used for transformation and the various transformation techniques used for plant ,algal ,and bacterial vaccines carriers . Edible vaccines can be very easily scaled up. for example, the entire population of china could be vaccinated by producing edible vaccines in just 40 hectares of land .Chance of contamination by plant pathogens is very low or rather in significant as plant pathogens are not capable infecting human beings. [11]Edible vaccines against various diseases such as measles , Cholera, foot and mouth diseases, and hepatitis b ,C and E are produced in plants like banana ,tobacco, potato etc ..,[12]

**EDIBLE PLANT VACCINE**:In the late 1980's the use of plants has a recombinant expression systems began to receive attention. Over mammalian expression systems, plants have a significant advantage in that they are powered by photosynthesis, [13,14]which eliminates the need for an external carbon supply. The lack of pathogen contamination in the plant system over a mammalian system is another significant advantage. The production of antigens, vaccines, and other eukaryotic proteins in plants is particularly made more interesting by these benefits. [15,16]

**EDIBLE ALGAL VACCINE:**Algal edible vaccines are similar to plant edible vaccine . algae are sometimes reffered to as single- celled water – borne plants.There are very few strains of algae athart are considered edible for human beings and are capable of being genetically enginerred to deliver antigens against various diseases. The usage of algae has many advantages such as :

- Micro algae are much easier to be genetically modified , thus showing higher expression level of foreign genes [17]
- Algal vaccines are relatively cheaper compared to these produced by plants .
- Algae are potential source of food for many species including humans .[18]
- Micro algae are resistant to animal pathogens, thus making them a very good mode of vaccine production.

**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[19]. 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[20]. The complete unfused recombinant protein that accumulates with in the plants is expressed by polypeptide expression systems.[21]

Edible vaccines are subunit vaccines : they contain proteins for a pathogen to form .[22,23] The first steps in making an edible vaccine is the identification , isolation and characterization of a pathogenic antigen.[24] 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 . It is then inserted in to the genome , expressed , and inherited in a mendelian fashion , which results in antigen being expressed in fruit or plant. From that point forward , traditional vegetative methods and techniques are used to grow the plants and propagate the genetic line.

**MAJOR PLANT SPECIES USED AS A VACCINE:** Rice is the other plant species that has been employed in the creation of edible vaccinations . benefits over the other plants included being regularly used in infant food and having a high antigen expression level . However ,it develops slowly and necessitates the use of glass house vaccines made from rice plants will have a huge effect on the health in areas where the rice is an important food source.[25,26]**POTATO**It is good model for developing vaccines for Hepatitis B, diphtheria, tetanus, and Norwalk virus. In humans, potato may serve as an oral strengthening against for hepatitis B vaccines.[27] The fundamental advantage of making edible vaccines from potatoes is the simplicity with which they may be transformed and propagated. Refrigerators are not required for storage, and one of the main disadvantages is that heating causes antigens to denature. [28]**TOMATO**An effective vaccine against acute respiratory illness, SARS was developed in tomato. It has a better antiviral effect against the Norwalk virus.[29] Tomatoes were used to create vaccinations for septicemia, pneumonia, and the bubonic plague. It grows swiftly and may be cultivated in a wide range of environments. Tomatoes include a lot of vitamin A, which may help your immune system. It, other hand, quickly spoils.[30,31]

**CARROTS**Carrots are not only nutritious and tasty, but they may also be used to make edible vaccinations. When created in transgenic carrots, vaccines against E .coli Helicobacter pylori and HIV indicate potential effects. Consumption of this sort of antigen-containing carrot consumable vaccine benefits those with weakened immune systems.[32,33]

**CURRENT STATUS AND APPLICATIONS :** The evolution and marketing of edible vaccines takes time and patience. Many edible vaccines have been produced for animal and human illness, and have progressed through various stages of clinical testing. Numerous clinical trials were carried out to validate the vaccines' opportunities for human ingestion. Several communicable diseases in humans and animals, including hepatitis B, measles, and cholera, have been studied for edible vaccines.[34,35] The discovery of effective and economical medicinal chemicals in transgenic plants sparked significant developments in medical research and plant biology. Since 1986, many pharmaceutical therapeutic proteins, antigens, antibodies, monomers, enzymes, hormones, and growth regulators have been produced in various plants such as tobacco, banana, tomato, carrots, rice, maize, lettuce, Alfalfa, potatoes, peanuts, spinach, apple, papaya, bean (viciafaba), Arabidopsis, soyabeans, and clover as edible vaccines against various diseases with various purposes.[36,37] Which are given below:**Malaria** is one of the leading causes of morbidity and mortality in the globe, with 300 to 500 million new infected individuals each year, resulting in 1.5 to 2.7 million fatalities. Merozoite surface protein (MSO) 4 and MSP 5 from plasmodium falciparum, as well as MSP 4/5 from p.yoelli, are now being studied for the creation of plant-based malaria vaccine.[38]**Cholera**Transgenic potatoes containing the CT-B gene of vibrio cholera have been demonstrated to be effective in mice. It was claimed that eating one potato every week for a month, along with occasional boosters, would offer immunity. The co-utterance of mutant cholera toxin subunit A (Mct-a) and LT-B in agricultural seed has been proven to be successful and feasible via nasal delivery.[39,40]

**Hepatitis:** Hepatitis according to WHO projections, two billion individuals. Over 360 million people are chronically infected with HBV, and over 600,000 people die from HBV-related illness such as liver cirrhosis or hepatocellular carcinoma. HBsAg, or hepatitis B surface antigen, is employed in the manufacturing of edible hepatitis B vaccination. Potatoes are the plant of choice for the creation of an edible hepatitis vaccine. The expression of HBsAg is more prevalent in the roots than in other regions of the plant.[41,42,43,44]

**Measles:** Every year, measles kills 800,000 people worldwide. The measles live attenuated vaccine (LAV) has no oral effectiveness and is destroyed if a cold chain of refrigeration is not maintained. The presence of maternal antibodies in the LAV decreases its efficacy. There are two surface protiens, hemagglutinin (H) and fusion protiens, with H protein contaminated with wild-type measles virus. The results showed that IgA antibodies were present in the faeces of animals vaccinated with MV-H. According to research, transgenic carrot plants are the wisest option for measles immunizations.[45,46] Mice fed tobacco that produced MV-H (Edmonston strain measles virus heamagglutinin) had antibody titers five times higher than What is considered effective and preventing for humans, as well as secretory IgA in their excrement.[45]

**Anthrax** The possibility of using Bacillus anthrax is as a bioweapon has increased the urgency of establishing a vaccination against it. Tobacco leaves inundated with the pag gene (anthrax protection antigen-PA) using a gene gun might produce a protein that is physically equivalent to the main protein found in the current vaccine. Anthrax antigen might be manufactured in billions of units. Furthermore, this vaccination lackedtheedoema and fatal factors that were accountable for the toxic side effects. Tomato plants are now being inoculated with the same anthrax antigen. Scientists are also attempting to convert spinach by transfecting it with TMV-expressing PA, in the hope that spinach will be a safer vaccine.[47,48]

**Rabies** Antibodies against rabies might be induced in mice by tomato plants producing rabies antigens. TMV can also be used as an alternative. CaMV-transformed tomato plants bearing the rabies virus (ERA strain) glycoprotein (G-protien) gene were shown to be biologically active in mammals.[49]

**Diarrhoea** is the third biggest cause of death among Indian children. The most common originator of diarrhea is GIT infection. The pathogens that caused the illness included bacteria, viruses, and parasitic organisms. Although several oral vaccinations have been produced for the prevention of diarrhea, only a few musical active vaccines targeting pathogens have been licensed. To be effective, oral vaccination must travel through the hostile environment of the stomach and intestine. This can be accomplished by designing edible vaccines against enterotoxigenicEscheichia coli (ETEC), cholera, and norovirus. It used Agrobacterium tumifaciens to transfer gene-encoding LT B to tobacco and potato leaves, which were then given to mice. Mice fed these potatoes and tobacco leaves generated blood IgG and mucosal IgA anti-LT-B antibodies [50]

**Cancer therapy** Certain plants have been efficiently designed to produce monoclonal antibodies, which have been shown to be useful cancer therapeutic agents. In the case of soyabeans, foe example, Monoclonal body (BR-96) is a powerful antidote for the drug doxorubicin, which develops ovarian cancer, breast cancer, lung cancer, and colon tumours.[51]

**Role in autoimmune diseases** In terms of autoimmune diseases, research into boosting self-antigen production in plants is still in its early phase. Among the illness being studied include multiple sclerosis, rheumatoid arthritis, lupus, and transplant rejection. Diabetic mice were fed potatoes capable of generating insulin as well as protein called GAD (glutamic acid decarboxylase), which was linked to the CT-B monomer in one clinical investigation. The protein was revealed to be efficient in lowering immunological assaults and delaying the onset of hidh blood sugar levels.[52]

**Rotavirus** is responsible for 25% of diarrhoea-related fatalities in underdeveloped nations. A tumafaciens converted the vector to potato by fusing the rotavirus VP7 glycoprotein to the endoplasmic reticulum transporter SEKDEL gene. Mice were given potato tubers, which evoked blood IgG and mucosal IgA response against the virus.[53,54]

**Treatment of covid-19** Corona virus (COVs) are a diverse category of positivesense implanted RNA viruses with genomes ranging from 27 to 32 kb.[55] Around 20 days after the SARS-Cov-2 genetic sequence, Medicago , a Canadian biopharmaceuticals company, succeed in producing virus like Particles (VLPs) of the corona virus. Despite using egg-based vaccine Production methods, this methodology involves inserting an encoded Genetic sequence of COVID 19 spike protein into agro bacterium, a common soil bacterium that is then ingested by plants.[56]

## II. LIMITATIONS AND CHALLENGES :

While the concept of edible immunization is enticing, putting them into action can be difficult. Many difficulties must be addressed in order to produce a plant-based vaccination, including selection of antigens, dose, quality control, selection of plant, conveyance, efficacy, safety, public perception, and licencing.[57] Antigen selection raises the question of whether chosen antigens are suitable enough with the plant type to be expressed safely. The weight, age, and size of the fruit or plant, as well as the ripeness of the fruit or plant, all influence the dosage.[58]Because no two potatoes or bananas are the same size, considerable variances in protein content may occur. This could lead to the risk of underdosing, which would result in tolerance. As a result, dose consistency from any fruit to fruit, plant to plant, and generation to generation is a concern.[59]Plant crops must have a long shelf life. Because these fruits are employed as vaccine vectors, they must be maintained correctly to minimise infection or sickness due to spoiling.[60] Another issue to consider is transgene escape and identifying the “vaccine” fruit from a regular fruit to avoid vaccination misadministration.[59] excess mRNA may be introduced into the plant genome as a result of methods used to increase the antigenic protein concentration in transgenic plants by stunting plant growth and reducing fruit production.[57] Furthermore, plant-based vaccinations may cause an allergic reaction or other side effects such as cytokine-induced illness, central nervous system damage, or autoimmune diseases. The issue is to make the procedures easier to follow without sacrificing quality, which is a requirement for producing plant-based edible vaccines.[61]

## III. FUTURE PROSPECTIVE:

Although edible vaccinations are not yet available, researchers in field as diverse as agricultural and biotechnology make it feasible to imagine that the a toddler being vaccinated while eating a tomato,[62] is not far-fetched .In concept, it is now feasible to transfer an organism’s gene to any plant and have that gene express a new product in any part of the plant, be it the seed, leaf, root, or tuber. Food is increasingly being seen not just as a fundamental source of nutrition, but also a product with distinct medical benefits. Many factors influence the future of edible vaccines. It must be well accepted by the general public, thus society must be educated on the use and benefits of edible vaccinations. The stability of genetically engineered plants is the next key bench mark to assess, and proper plant isolation is required.Future research and development on edible vaccines will evaluate if these vaccines can meet WHO quality standards, such as safety, potency, efficacy, and purity. Most diseases would be able to be vaccinated worldwide if these vaccinations become a reality.

## IV. DISCUSSION :

Edible vaccines hold a great advantage as a cost effective, easy to administer, easy to store, fail safe &socio culturally readily acceptable vaccine delivery system ,especially for the poor developing countries. It involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. It has been a concept about a decade ago , it has became a reality today .A variety of delivery systems have been developed and patents have been applied. Edible vaccines are currently being developed for several human & animal diseases. There is growing acceptance of transgenic crops in both industrial as well as developing countries. Resistance to genetically modified foods might affect the future of edible vaccines. They have passed the major hurdles in the path of an emerging vaccine technology. Various technical obstacle, regulatory& non- scientific challenges were

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observed. The use of edible vaccines has different advantages & disadvantages. Therefore, the edible vaccine in form of fruit vegetable can be sold to an affordable price. However, the cost of injectable vaccines against diphtheria, tetanus. They are so cheap now that there would be no reason to develop edible vaccines against these disease . But on the other hand, the use of syringes for vaccination can cause infections due to possible contamination of needles that have been used before. The risk may seem small but it is an upcoming problem in developing countries and could be minimized by using edible vaccines. One of the disadvantage was the difficulty to control the dosage of the vaccine. The question is whether a high dosage will provoke oral tolerance of an invading bacteria or virus instead of an immune response. The dosage, which is needed for adults is also very different from the dosage needed for children. To determine the correct dosage, one needs to know the persons weight , age, the fruits/plants size, ripeness & protein content. In addition to that,the oral intake of vaccines is not convenient for infants and therefore it is probably better to concentrate the vaccine dosage in one teaspoon of baby food rather than in whole fruit. It is a prevention of diseases instead of a cure. The oral mucosa is the body's first line of defence against different bacteria. Experiments with humans have only shown promising results but it is still unknown what will happen if the subject comes in contact with the actual virus. If you cross two plants, which produce different vaccines, you will be able to get a plant, which produces more than only one antigenic protein. This can lead to combination effect of vaccines and would be highly useful in developing countries like in Africa.

## V. 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. For example trials of edible vaccines against hepatitis B have reported encouraging results but it will still take time until this concept finds its way to the market because further research is needed. 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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