IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

An overview On Edible Vaccines: Need Of Future

Saurabh Nandkumar Ahire*, Rutuja Tukaram Panchave, Aishwrya Balu Patil.

Dr. D. Y. Patil College Of Pharmacy Akurdi, Pune.

1. Abstract:

Vaccines comes under the branch of biotechnology which are one of the most important tools used worldwide for prevention of life threatening infectious diseases**.** Although, the major problem for the oral immunization is the digestion of macromolecule antigenic protein inside the stomach because of extremely acidic pH. To solve this problem, scientist Arntzen developed the theory of edible vaccines (EVs). Edible vaccines are prepared by exposing the selected desired genes into the plants and then using these altered plants for the production of encoded proteins. The altered plants are known as ‘’transgenic plants’’ and the process is called as ‘’transformation’’ in terms of biotechnology. Edible vaccines are generally GM crops which provide immunity against the diseases such as HIV, cancer, Hep. B, pneumonia. The main strategies based upon edible vaccines formulations has been demonstrated to increase both systemic as well as mucosal immune response. This novel vaccination delivery systems having various advantages over injectable vaccination preparations including stability, self-administration, reduced cost, and elimination of cold chain.

In this review the latest findings related to edible vaccines are discussed including the mechanism of action, preparation methods, applications, advantages, disadvantages, limitations, and future of edible vaccines is also discussed.

Keywords: Transgenic plants, edible vaccines, vaccines, immunity

2. Introduction:

Vaccines are comes under the branch of biotechnology which supply protection against various infectious diseases and disorders. All organisms are vulnerable to one or more forms of infectious and non-infectious diseases throughout their life. To prevent these infection researchers discovered plant-based vaccine which is an immune-biological substance, used for specific protection against both infectious and non-infectious diseases.The process of distributing and administrating vaccines is referred to as vaccination and vaccination is the form of immunization [1-4]. Efficacy, safety and cost of existing vaccines are major factors related with vaccine production, Distribution and availability. Therefore, it becomes essential to produce new vaccine that is more efficacious, safe and cost effective in comparison to the existing vaccines for the benefit of common people. Hence, an alternative and new approach is the preparation and use of edible vaccines is come into the picture now a day. As per the literature reports available, plant viruses can be genetically engineered to produce vaccines against diseases such as cholera and life-threatening infections like AIDS, chicken pox etc., [5,6]. In addition to this, plants and their edible products provide an agreeable alternative for the synthesis of the recombinant proteins in a large-scale production process [7]. The first report of edible vaccine (a surface protein from streptococcus) in tobacco, at 0.02% of total leaf protein level, appeared in 1990 [8]. The theory of edible vaccines got success after Arntzen and co-workers expressed hepatitis B surface antigen in tobacco plants [9]. Multicomponent vaccines were also developed which are the second-generation edible vaccines that provide protection against several disease-causing pathogens [10].

2.1 Concept of Edible Vaccines:

Edible Vaccines can be developed by incorporating the selected desired genes within the plants and then this genetically modified plants allowed to supply encoded proteins, the process in terms of molecular biology referred as transformation process, for that reason the altered plants are known as transgenic plants. Traditional subunit vaccines having an advantage that they contain antigenic protein but the traditional vaccines are having high cost so can’t afford easily and technology required for the production is also complex Edible vaccines also contain that antigenic proteins, these vaccines are act by stimulating the mucosal also systemic immunity when they comes in contact with alimentary canal lining.This dual mechanism of action of edible vaccines gives first-line defense against pathogens attacking through mucosa, such as tubercle bacillus and carriers causingHIV, diarrhea, STDs, pneumonia, etc [11]. Preparation of edible vaccines is effective process and may be easily scaled up. Edible vaccines offer numerous advantages like they possess good genetic and warmth stability and don't need cold-chain maintenance. Long-distance transportation ofedible vaccines is also avoided by storing it at the location of use. Unlikely other vaccines, in edible vaccines there is no need of trained medical personnel as there is oral administration particularly in children. Syringes and needles also are not required, thus decreases the incidence of various infections [12]. Mothers who are administering the edible vaccines orally might be useful to immunizing the fetus in uterus through placental movement of maternal antibodies or in the new born baby through the breast feeding. Edible vaccines enable the tactic of seroconversion within the presence of maternal antibodies, thus playing a possible role in protecting children against diseases like group-B Streptococcus, respiratory syncytial virus (RSV), etc [13].

2.2 Ideal properties of an edible vaccine:

i. It should be neither toxic nor pathogenic, i.e., it should be safe to administer to human being.
ii. The vaccination technique should be simple.
iii. The vaccine should be less cost and should be feasible to consumer.
iv. It should produce cellularand humoral immunities lasted for long period.
v. It should have very low levels or no side effects in normal individuals.
vi. It should not cause any problems in individuals with impaired immune system.
vii. Contamination of the environment should be avoided [14,15].

2.3 Advantages:

i. One of the main advantage of edible vaccines is it is very effective for delivering the immunization so the adjuvants which enhance the immune response in cancer patient are not necessary.
ii. Edible vaccine can elicit mucosal immunity which is not occurred in traditional vaccines.
iii. Edible vaccines are cost effective, easy to preparation, production, storage and transportation. Because edible vaccines are produced by biological method are stable at room temperature while traditional vaccines need cold storage facility which ultimately increases the yearly cost to preserve and storage of vaccines [16]. Moreover, the seeds of transgenic plants could be store for long term use as they contain less amount of moisture so easy to dry. For the production of edible vaccines there is no requirement of costly equipment and machines as they could be easily made on soil rich land and the cost for growing plants is also low as compare to cell culture grown in fermenter.
iv. As edible vaccines are produced from plants, they are easily available. Manufacturing cost is low as there is no need of special premises for manufacturing them and facilities like sterilization as required in traditi onal vaccines [17].
v. Edible vaccines are easily accepted by the patients as the don't require to administer by the injection. So, there is also decreases the requirement of personnel to administer the dose of vaccines, lessen the chances of contamination because the sterile conditions are not required [18].

2.4 Disadvantages:

i. It is difficult to finding and selection of particular plant with stable antigen production, expensive and time-consuming process.
ii. Due to insufficient knowledge related to plant biotechnology leads to negative public point of view towards edible vaccines, strict laws 7 regulations and debates related to intellectual property discourage pharma business investment in edible vaccines.
iii. There may be chances of hypersensitivity reaction, development of oral tolerance to the vaccines and difficulty in administration of standard dose are some limitations [19,20].
iv. Edible vaccines are depending on stability of plant as some fruits like potatoes cannot eaten directly. That means they need to cook before eating and this cooking can cause denaturation or weaken the proteins present in it [21].

v. Proper differentiation line is necessary between 'normal fruit' and 'vaccine fruit' to avoid misadministration of vaccine, which can lead to vaccine tolerance.

3. Mode of action of Edible vaccines:

Edible vaccine is a new way of immunization through oral route. This technique will have large contribution to the global vaccine program and might have a dramatic impact on health care sectors in developed and developing countries. Mostly the pathogens attack on mucosal surfaces and therefore, it is the most effective site for vaccination. Whenthese edible vaccines comes in contact with the digestive tract liningthey activate both systemic and mucosal immunity. This dual effect would offer first-line resistance against pathogens which are entering through mucosa.

Edible vaccines arethe type of mucosal-targeted vaccines, which stimulate both systemic and mucosal immune response. After administration of edible vaccines, they go inside the body tissues at the site of action where they enter through M cells. M cells passes the antigen from vaccine to macrophages and B cells. Then the macrophages expose the pieces of antigen to helper T cells.T cell stimulates B cells and search out the antigens at distant sites. These antibodiesactivate the B cells, this activated B cells produces antibodies. These produced antibodiesareused to neutralise the antigen. This whole process is the initial response of edible vaccines inside the body (as shown in fig. no. 1a) [22].

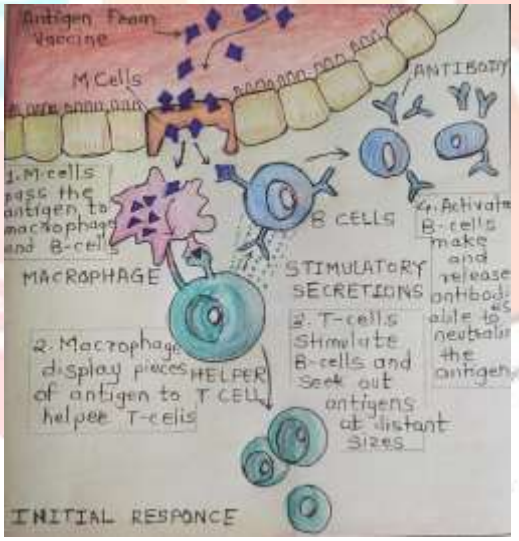

Fig. 1(a): Initial Responce

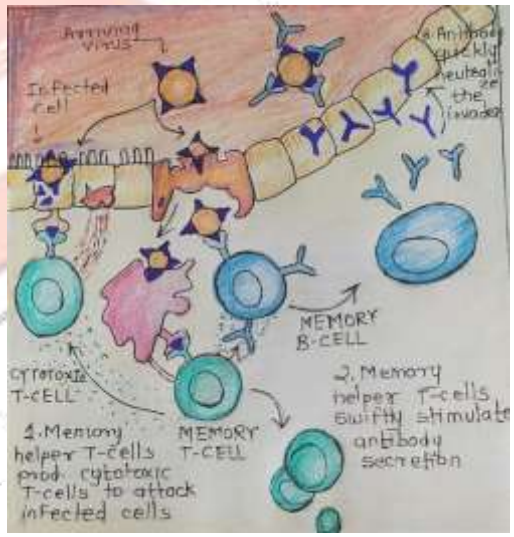

Fig. 1(b): When a disease agent appears

When a diseased agent appears the memory helper T cell produces cytotoxic T cells to attack infected cells, also these memory helper T cells immediately stimulate the secretion of antibodies. Then these antibodies immediately use to neutralize the invader antigens or virion.(as shown in fig. no. 1b) [22].

4. Preparation of Edible Vaccines:

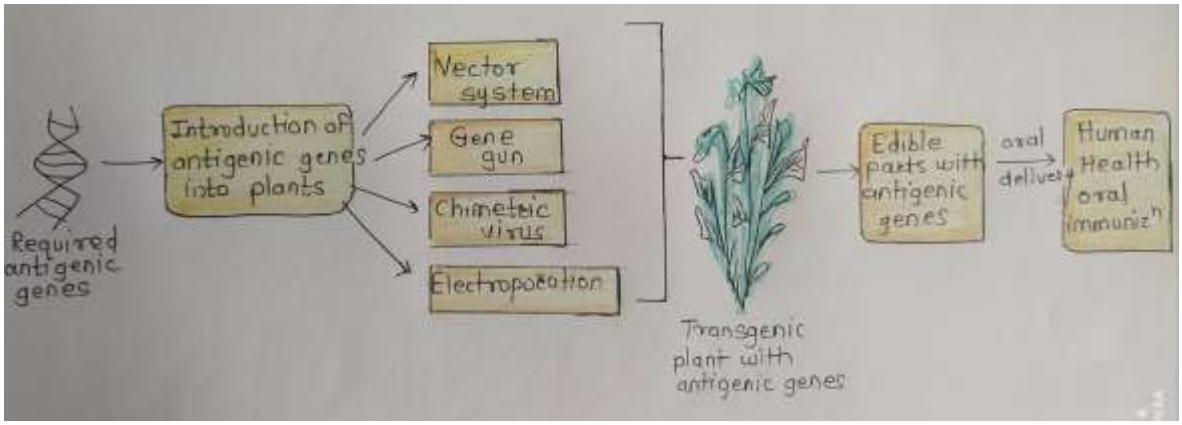

Fig. 2: Concept of abstract of edible vaccines

Following are the methods for transformation of DNA/ gene into the plant:

i. Plasmid /vector carrier system: *A. Tumefaciens* is a naturally occurring soil bacterium use to transfer small DNA segment into plant genome by the process of transformation. After that the whole plant is generated from individual plant cell. The existing studies showed that genes are successfully expressed in experimental plants and when it gives orally to animals, the transgenic plants extract containing antigen induces theproduction of serum antibodies in that animal [23-25]. The study revealed that *A. Tumefaciens* is used to produce edible vaccines. vegetable pathogens like *A. tumefaciens* and *A. Rhizogens* can integrate their DNA (T-DNA) with infected cells nuclear genome [26]. so, the exogenous genes are introduced into modified T-DNA of cells of *A. Tumefaciens* then infected to vegetable tissue which causes gene's stable integration in plant's genome and synthesis of transgenic protein. Initially agrobacterium mediated transformation was limited to tobacco and few other species which are infection's natural targets. But it has been now extended to most of the vegetable species of agronomic interest including Gramineae and Leguminosae family [25,27]. This extends the use of edible vaccines for human as well as veterinary use.

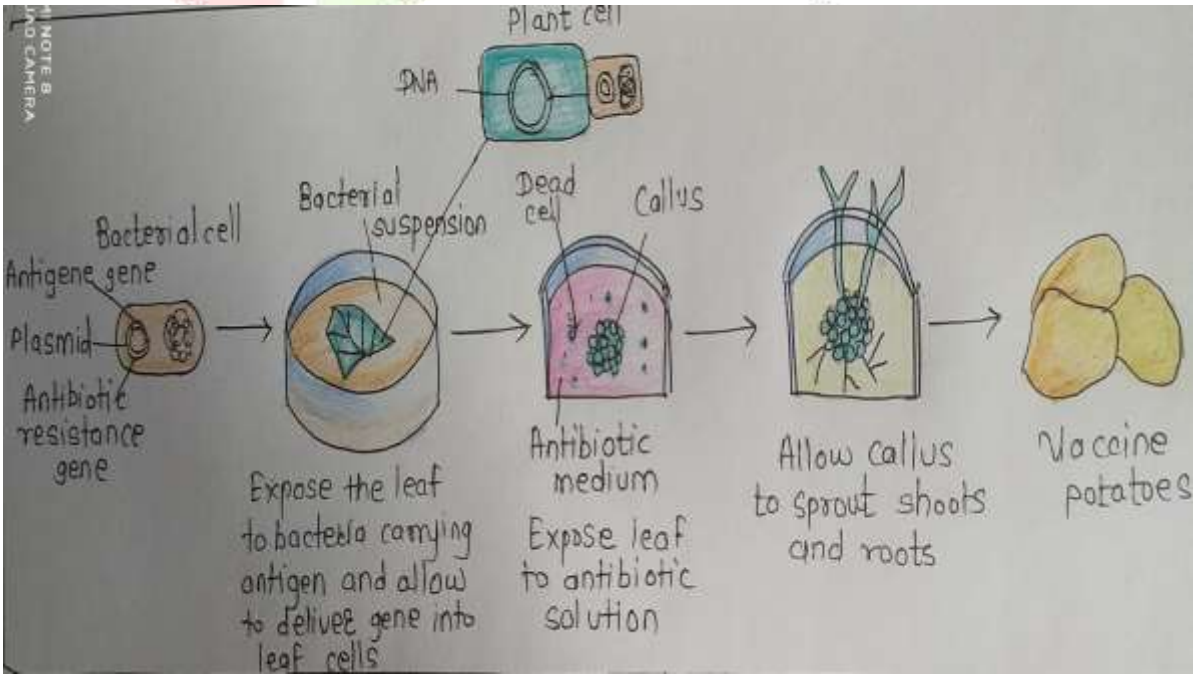

Fig. 3: Schematic representation of plasmid/vector carrier system

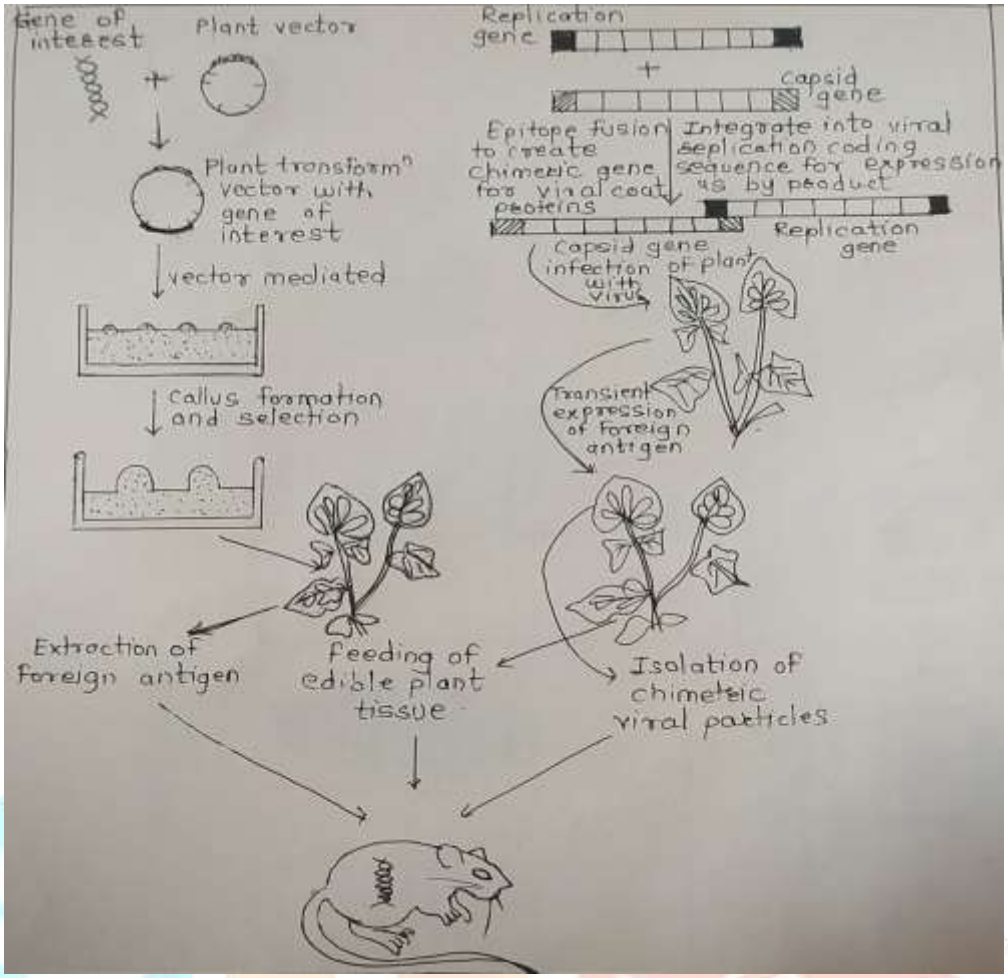

Fig. 4: Scheme for production of candidate vaccine antigens in plant tissues.

ii. Micro projectile bombardment (Biolistic) method: In this method selected DNA sequences are precipitated onto the metal microparticles and then they are bombarded on vegetable tissue with the help of particle gun at very high speed. This microparticles penetrates into the walls of vegetable tissue and release the exogenous DNA into the cell where it gets integrated. In this biolistic particle delivery system, Sufficiently processed DNA particles bombarded on the cell which then penetrate into chloroplast and start integrate with its genome. The chloroplast's transformation is an interesting alternative for nuclear transformation [28,29].

iii. Electroporation method: The DNA is introduced inside the cells by exposing cells for a short period to high voltage electrical pulse which is supposed to produce temporary pores inside the plasmalemma (cell membrane). The cell wall acts as barrier for the entry of DNA inside the plasmalemma. So, it has to be weakened by enzymatic treatment thus for the entry of DNA into the cell membrane [4,14].

iv. Chimeric virus method: Plant viruses are genetically modified to carry perticular genes and used to infect their natural hosts which is edible plants where the cloned genes expressed themselves to varying degrees in various edible parts of host plant. Particular viruses can be redesigned to express fragments of antigenic proteins on their surfaces like cauliflower mosaic virus (CaMV), alfalfa mosaic virus, tobacco mosaic virus, cowpea mosaic virus, tomato bushy stunt virus, and potato virus [4,29,30].

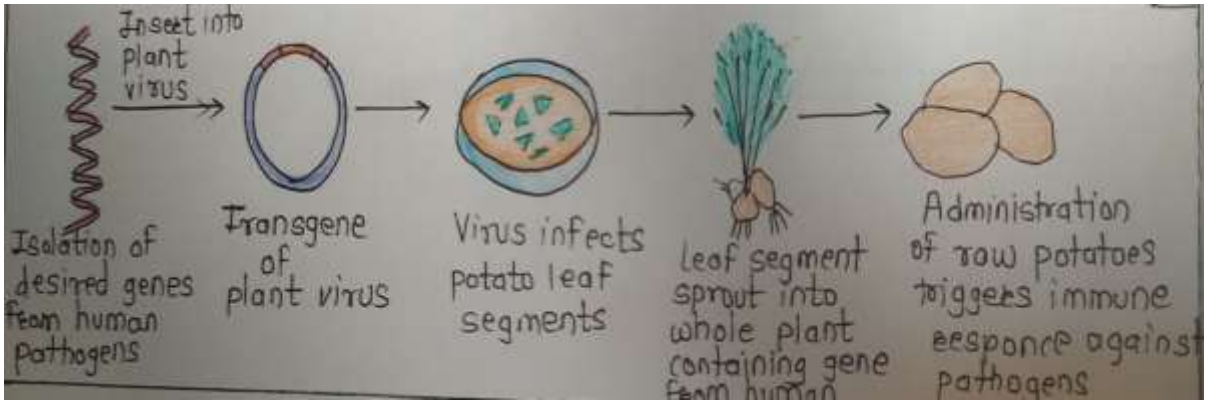

Fig. 5: schematic representation of chimetric virus method

5. Advantages, disadvantages of plants of fruits use for the preparation of edible vaccines [6] and their use in Table no. 1:

Plant/fruit	Advantages	Disadvantages	Virus
Tobacco	• Good model for recombinant proteins evaluation • Antibodies stored in the seeds can be easily purified at any location • numerous seeds, stored for long time requires low-cost preserving system • Large harvests, number of times/year	• May produces toxic compounds*	• Rabies virus[45] • Hep. B virus[45] • Dental caries[45] • Measles[6] • Enterotoxigenic *E. coli*[6] • Norwalk virus[6] • Cytomegalo virus [6] • Transmissible gastroenteritis coronavirus [6]
Potato	• Dominated clinical trials • Easily manipulated/transformed • Stored Easily for long duration without refrigeration	• Cooking is required. But it denature antigens and may decrease immunogenicity**	• Hep. B virus [45] • *Vibrio cholera*[45] • Norwalk virus [6] • Enterotoxigenic *E. coli* [6]
Banana	• Inexpensive • Do not need cooking • Grown widely in developing countries Proteins not destroyed even if cooked	• Maturation period is 2-3 years • Spoiling occurs after ripening • Transformed trees requires about 12 months to bear fruit • Contains very little amount of protein, so	• Cancer therapy [6]

		unlikely to produce large amounts of recombinant proteins	
Tomato	• Broadly Cultivated • Grow quickly, Heat-stable, made into capsules • Antigen-containing powders*** • High amount of vitamin A canincrease immune response • Spoilage problem Overcome by freeze-drying technology • Different batches blended to give uniform doses of antigen	• Spoils readily	• AIDS [45] • Rabies virus [6] • Hep E virus- E2
Rice	• Easy storage/transportation • Used in baby food due to low allergenic potential • Expressed protein is heat-stable • High expression of proteins/ antigens	• Grows slowly • Specialized glasshouse conditions required	• Treatment of cancer [45]
Lettuce	• Fast-growing • Direct consumption	• Spoils readily	• Hep. B virus [6]
Soybean and Alfalfa	• Large harvests, number of times/year		• Foot and mouth disease [6]
Musk melon (cantaloupe)	• Easily transformed • Fast growing • Easily propagated by seed		
Others	• Carrots, peanuts, wheat, corn		

*Currently, therapeutic proteins in tobacco are being produced.

**Some of South American potatoes can be eaten raw. However, some studies show that cooking does not destroy all complements of antigen in potatoes.

***Freeze-dried tomato powder consisting NV capsid and LT-B was found immunogenic. Similar technique is also used for potatoes and carrots.

6. Application:

i. Autoimmune disease: Scientist have determined that some cell proteins which can induceautoimmunity to the people which are suffering from Type I diabetes malleates. Then there is beginning of development of plant-based vaccines in tobacco and potatoes containing insulin or GAD linked to innocuous B subunits of *V. cholerae toxin* to increase uptake of antigen by M cells. When the transgenic potatoes and tobacco plants fed to non-obese mice with diabetes increases IgG antibody level which is associated with cytokines which suppress harmful immune responses. So, feeding of vaccines can cause suppression of autoimmune attack in the diabetic mice and may delay rise of blood sugar level [31-32].

ii. HIV: Initially HIV protein is spliced into CPMV successfully [33]. Then two HIV protein genes and CaMV as a promoter were successfully injected with the help of needle in tomatoes, and these expressed proteins are converted into multiple copies by polymerase chain reaction(PCR) technique in various parts of plants also include the ripe fruits in addition to second generation plant [34]. Recently, for the expression of Tat protein, these proteins are cloned into TMV with the help of inoculation of spinach. Each gram of spinach leaf tissue was containing up to 300-500 µg of Tat antigen [35]. When the mice is fed with this modified spinach followed by DNA vaccination inhigher amount antibodythan that of control, with the levels peaking at four weeks after vaccination.

iii. Gastrointestinal disorders: As per the WHO cholera vaccine can give cross protection against enterotoxin *E. coli* a heat labile enterotoxin (LT-B). When the transgenic potatoes expressing LT-B were fed to mice, these transgenic potatoes induce production of serum and secretory antibodies. Afterward, evidence also provided that cooking of raw potatoes does not cause inactivation of antigen present in edible vaccine. So, the spectrum of edible vaccines producing plant is expanded beyond the raw food like fruits, flowers, leaves [36-38].

iv. Measles: Live attenuated vaccine is the only currently available vaccines for measles but they are having certain disadvantages. For preparation of edible vaccine, with the help of plasmid vector MV-H antigen is introduced in tobacco plant. These edible vaccines produce serum antibodies which induce immune response against antigen. The IgA antibodies with antigen also found in the faecal samples of immunized animal [12,39].

v. Malaria: three mainly used antigen are used for the production of malaria vaccine which are Merozoite Surface Protein (MSP) 4, MSP 5, MSP 4/5. This recombinant antigen is orally given to mice along with CBT as mucosal adjuvant, produces immune response. However oral delivery of these malarial vaccine producing immune response is uncertain.

vi. Cholera: when the transgenic potato with CT-B gene of *vibrio cholerae* is given orally to mice will produce efficacious effect. Eating one potato per week consecutively for one month with periodic boosters is said to provide immunity [40]. Co expression of mutant cholera toxin subunit A(mCT-A)And LT-B in crop seed when administer by nasal route shows effective action and is practical [41].

vii. Rabies: Rabies antigens are expressed by tomato plants could induce antibodies in mice [33]. TMV is also used as an alternative [42]. Tomato plants is transformed by using CaMV and the glycoprotein gene of rabies virus (ERA strain) which is having immunogenic action in animals [43].

viii. Norwalk virus:When transgenic potatoes expressing Norwalk virus is administered by peoples, there are chances of development of seroconversion in 95% peoples (19 out of 20 peoples). Genetically engineered

bananas and powdered tomatoes expressing Norwalk virus are under development phase to combat Norwalk virus [44,45].

7. Future perspectives of edible vaccine:

The future of plant based edible vaccine technique is very bright as these vaccines provides safety, cost effectiveness, eliminate the storage requirement also requirement of trained medical practitioner for delivery of vaccine Edible vaccines can be manufacture in the bulk at low cost and also requires less processing time. This vaccines can prepared even at the site of utilization in very safe manner.As we all know that the traditional vaccines play very important role to improve immunity and making better health of humans but their bulk formulation is very time consuming and costly, so, edible vaccine technique is another approach as an alternative to sort out these issues. By using this technique more effective, better and safer immunization in addition to prevention of disease can be given to the peoples specially for life threatening disease affect bigger masses of population globally such as dengue, AIDS, heart disease, intestinal diseases, malaria, various cancers, respiratory infections, diseases and disorders. Furthermore, in future, multi component vaccines as an adjuvant can be developed by crossing of two generation of plants harbouring various types of genes expressing clinically important antigens [46,47].

The future of edible vaccine also depends on the WHO standards especially on the safety, purity, effectiveness, cost and with more efficiency [48]. The acceptance and development of transgenic crops at larger scale is also a concern in many developing countries, and if this is accepted by the society then production of edible vaccines and vaccination for the various diseases will be possible worldwide [6,49]. Presently, microalgae are being used as very valuable and important source which providing active molecules such as chlorophyll, fatty acids, carotenoids, phycobiliproteins and enzymes. Microalgae can be used in future for the recombinant protein expression, pharmaceuticals, immune regulators, hormones, growth factors, nutraceuticals, and many other products such as anticancer agent Taxol and they can be used as edible vaccine. They have many advantages such as formulated at large scale production and fast transformation and fast growth and stable expression level with proper folding and accumulation of multiple antigens as vaccines. They also have effective delivery through oral route. they can be converted into tablets or capsules formulation to ease the administration and immunogenic response [50-52].

8. Conclusion:

Edible vaccines are very safe and at cheaper cost alternatives to the traditional vaccines and it is a novel approach to oral immunization. Any edible plant or algae they can make scaling up so much easier. Limitations associated with traditional vaccines such as productions, distribution, delivery can be eliminated by replacing them with edible vaccines through various immunization programmes. In future there may be increase in the need of safer, economical and efficient delivery system to be developed at large scale in the form of edible vaccines. Edible vaccines provide a great opportunity in the future where there will be no any injectable needles are used but a different way may be available where an individual get protection against the diseases and disorders by simply eating the fruits.

9. References:

1. Charmi PS, Manisha NT, Urmila DV, Vishwash JJ. Edible vaccine: A better way for immunization. Int J Curr Pharm Res 2011; 3:53-56.
2. Morr TS, Gomez LM, Palmer KE. Edible vaccines: A concept comes of age. Trends Microbiol.1998; 6:449-453.
3. Daniel H, Streatfield SJ, Wycoff K. Medical molecular farming: Production of antibodies, biopharmaceuticals and edible vaccines in plants. Trends Plant Sci.2001; 6:219-226.
4. Hafiz E, Eyob H. Review on edible vaccine. Acad J Nutr.2015; 4:40-49.
5. Moffatt AS. Exploring transgenic plants as a new vaccine source. Science.1995; 268:658-660.
6. P Lal, VG Ramachandran, R Goyal, R Sharma. Edible vaccines: Status and future.Indian Journal of Medical Microbiology.2007; 25:93-102.
7. Schuyler S. Korban, Sergei F. Krasnyanski and Dennis E. Buetow. Foods as Production and Delivery Vehicles for Human vaccines. Journal of the American College of Nutrition. 2002; 21:212S-217S
8. Mason HS and Arntzen CJ. Transgenic plant as vaccine production system.Trends Biotechnol. 1995;13: 388-392.
9. Expression of hepatitis B surface antigen in transgenic plants. Proct Natl Acad Sci USA.1992; 89:11745-11749
10. Yu J and Langridge WH. A plant based multicomponent vaccine protects mice from enteric diseases.Nat Biotechnol.2001;19: 548-552.
11. Langridge WH. Edible vaccines. Sci Am.2000; 283:66-71.
12. Webster DE, Thomas MC, Strugnell RA, Dry IB, Wesselingh SL. Appetising solutions: an edible vaccine for measles. Med J Aust. 2002; 176:434-437.
13. Giddings G, Allison G, Brooks D, Carter A. Transgenic plants as factories for biopharmaceuticals. Nat Biotechnol. 2000; 18:1151-1155.
14. Singh BD. Biotechnology. Kalyani Publishers. 1998;1
15. Madhumita N, Deepak V, Pallavi U. Edible vaccines - A review. Int J Pharmacother. 2014; 4:58-61.
16. Nochi T, Takagi H, Yuki Y, Yang L, Masumura T, Mejima M, *et al*.Rice based mucosal vaccine as a global strategy for cold chain and needle free vaccination. Proc Natl Acad Sci USA. 2007; 104;10986-10991
17. Pascual DW. Vaccines are for dinner. Proc Natl Acad Sci USA.2007; 104:10757-10758.
18. Streatfield S J. Mucosal immunization using recombinant plant based oral vaccines. Methods. 2006;38:150-57.
19. Krishna C, Jonnala UK, Sri R. Edible vaccines. Sriramachandra J Med. 2006; 1:33-34.
20. Jacob SS, Cherian S, Sumithra TG, Raina OK, Sankar M. Edible vaccines against veterinary parasitic diseases – Current status and future prospects. Vaccine. 2013;31:1879-1885.
21. Moss WJ, Cutts F, Griffin DE. Implications of the human immunodeficiency virus epidemic for control and eradication of measles. Clin Infect Dis. 1999; 29:106-112.
22. Jayaraman R, Gosula S S, Haritha T, M Anitha. An Overview on Edible Vaccines: A Novel Approach to Oral Immunization. International Journal of Research and Innovation in Applied Science. 2018 ;3:8-14
23. Mariotte D, Fontana G S and Santini L. Genetic transformation of grain legumes: Phaseolus Vulgaris L. and P. coccines. J Genet Breed.1989; 43:77-82.
24. Chikwamba R, Cunnic J, Hathway D, MacMurray J, Mason H. A functional antigen In a practical crop: LT-B producing maize protects mice against *Escherichia coli* heat Liable enterotoxin (LT) and Cholera toxin (CT).Transgenic Res. 2002; 11:479-493.
25. Mercenier A, Wiedermann U & Breitender H. Edible genetically modified microorganism and plants for improved health.Curr Opin Biotechnol. 2001; 12:510-515
26. De La Riva GA, Gonzalez-Cabrera J, Vasquez-Padron R & Ayra-Pardo C, *Agrobacterium tumefaciens*: A natural tool for plant transformation. Electronic J Biotechnol. 1998;1:118-132.
27. Lee RWH, Strommer J, Hodgins D, Shewen PE, Niu Y,*et al*. Towards development of anedible vaccine against bovine pneumatic pasteurellosis using transgenic white cloverexpressinga Mannheimia Fusion Protein. Infect immun. 2001;69:5786-5793.
28. Daniell H, Khan MS & Allison L. Milestone in the Chloroplast genetic engineering: An environmentally friendly era in biotechnology.Trends Plant Sci. 2002; 7:84-91.

29. Maliga T. Engineering the plastid genome of higher plants.Curr opin plan biol. 2002; 5:164-172
30. Ramshaw IA, Ramsay AJ. The prime-boost strategy: Exciting prospects for improved vaccination. Immunol Today 2000;21:163-165.
31. Ma JKC, Hiatt A, Hein M. *et al*. Generation and assembly of secretory antibodies in plants. Sciences. 1995; 268:716-719.
32. Travis J. Scientists harvest antibodies from plants. Science News. 1998; 5:359.
33. Prakash CS. Edible vaccines and antibody producing plants. Biotechnol Develop Monitor. 1996; 27:10-13.
34. Available from: http://www.sciencedaily.com/ releases/2004/08/040816001548.htm.
35. Karasev AV, Foulke S, Wellens C, Rich A, Shon KJ, Zwierzynski L, *et al*. Plant based HIV-1 vaccine candidate: Tat protein produced in spinach. 2005;23:1875-80.
36. Mason HS, Haq FA, Clement JD & Arntzen CJ. Edible vaccine protects mice against *Escherichia Coli* heat labile enterotoxin (LT): Potatoes expressing a synthetic LT-B Gene. Vaccine. 1998; 16:1336-1343.
37. Leben M, Johansson S, Osek J, Lindblad M & Holmgren J, Large-scale production of Vibrio *cholera* toxin B subunit for use in oral vaccines. Bio/Technology. 1993; 11:1574-1578.
38. Richter L, Mason H S, Arntzen C J. Transgenic plants created for oral immunization against diarrhoeal diseas es. J Travel Med. 1996; 3:52
39. Webster DE, Cooney ML, Huang Z, Drew DR, Ramshaw IA, Dry IB, *et al*. Successful boosting of a DNA measles immunization with an oral plant-derived measles virus vaccine. J Virol 2002;76:7910-2.
40. Available from: http://www.isb.vt.edu/articles/apr9803.htm.
41. Yuki Y, Kiyono H. New generation of mucosal adjuvants for the induction of protective immunity. Rev MedVirol 2003; 13:293-310.
42. Available from: http://www.brown.edu/Courses/Bio_160/ Projects1999/rabies/vacc.html.
43. Available from: http://www.geocities.com/plantvaccines/ futureprospects.htm.
44. Tacket CO, Mason HS, Losonsky G, Clements JD, Levine MM, Arntzen CJ. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. Nat Med 1998;4(5):607-9.
45. Charmi PS, Manisha NT, Urmila DV, Vishwas JJ. Edible vaccine: A better way for immunization. Int J Curr Pharm Res. 2011;3:52-56
46. Akhilesh T, Anjali K. Edible vaccines: Let thy food be thy medicine. International Journal of Pharmacological Screening Methods. 2014; 4:105-108.
47. Anirban S, Navneet KG. Edible vaccines: Future and its prospects. International Journal of Ayurveda and Pharma Research. 2015; 3:36-44.
48. 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; 21:803-808.
49. Sharma M, Sood B. A banana or a syringe: journey to edible vaccines. World J Microbiol Biotechnol. 2010; 27:471-477.
50. Pecht E A, Mayfield S P. Algae-based oral recombinant vaccines. Front Microbiol. 2014; 17:60.
51. Yan N, Fan C,Chen Y, Hu Z. The Potential for Microalgae as Bioreactors to Produce Pharmaceuticals. Int J Mol Sci. 2016; 17:17
52. Naeema J, Fouzia S, Omar bin H, Khalid M, Shuaib M D, Ishrat M and Nayik G A. An Overview on Edible Vaccines and Immunization. Austin J Nutri Food Sci. 2016; 4: 1-5