



Review article

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## Tobacco plant: A possible key to Ebola vaccine

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### ABSTRACT

In 2014, the World Health Organization was notified of an outbreak of a communicable disease characterized by fever, severe diarrhea, vomiting, and a high fatality rate in Guinea. Virologic investigation identified *Zaire ebolavirus* (EBOV) as the causative agent. Approximately 30 years after the first epidemic, there is no vaccine or therapeutic medication approved to counter the Ebola virus. In this review, it emphasized that plant based antibodies can be an effective cure to Ebola hemorrhagic fever. Genetically modified *Nicotiana benthamiana* is used to manufacture ZMapp drug by Kentucky BioProcessing. ZMapp three-antibody cocktail binds to the Ebola virus to deactivate it and provides simulated immune response against sugar-tagged proteins on the outside of the Ebola virus, which had verified efficacy against Ebola infection in nonhuman primates and also successfully treated two American medical workers infected with Ebola virus disease.

## 1. Introduction

Ebola viruses, belong to the family Filoviridae, are considered as one of the world's most virulent pathogen which cause Ebola hemorrhagic fever (EHF) in human and nonhuman primates[1]. Virologic investigation identified *Zaire ebolavirus* (EBOV) as the causative agent (Figure 1)[2]. So far the epidemiology and ecology of this deadly pathogen is mainly unknown[3]. Unfortunately, these viruses have been unnoticed in unidentified reservoir species in sub-Saharan Africa, most probably as enzootic infections, until varying ecological circumstances leading to human infections[4-7].

The recent outbreak of Ebola has caused more cases and deaths than any previous Ebola virus disease epidemic. The World Health Organization (WHO) declared the epidemic to be a Public Health Emergency of International Concern on August 8, 2014[8,9]. Recently ZMapp serum is under development to fight against Ebola virus disease and tobacco plants is much in news as ZMapp drug

can be manufactured by using them[10]. Present review briefly describes the role of tobacco plant in medicine, history of medicinal use of tobacco and most importantly production of ZMapp drug through tobacco plant.



**Figure 1.** An electron micrograph of Ebola virus particle[2].

## 2. 2014 Ebola outbreak

The 2014 Ebola outbreak started in Guinea in December 2013 and now up to November 2014 (Figure 2)[11]. There have been 14413

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reported Ebola cases in eight countries since the outbreak began, with 5 177 reported deaths. Guinea, Liberia, and Sierra Leone are the countries with widespread and intense transmission while Mali, Nigeria, Senegal, Spain, and the United States of America are the countries with localized transmission of Ebola (Table 1)[13]. Ebola virus disease is linked with average fatality rate of around 50%, however in past outbreaks case fatality rates have varied from 25% to 90%. No proven treatment for Ebola virus disease is available up till now, but series of possible management including immune therapies and drug are presently being assessed[10].



**Figure 2.** Map of Guinea showing initial locations of the outbreak of Ebola virus disease.

The area of the outbreak is highlighted in red. The main road between the outbreak area and Conakry, the capital of Guinea, is also shown. The map was modified from a United Nations map[12].

**Table 1**

Confirmed, probable, and suspected cases in countries with widespread and localized transmission of Ebola virus[13].

Country	Case definition	Cumulative cases	Cumulative deaths
Guinea	Confirmed	1 647	958
	Probable	208	208
	Suspected	64	0
	All	1 919	1 166
Liberia	Confirmed	2 562	–
	Probable	1 716	–
	Suspected	2 600	–
	All	6 878	2 812
Sierra Leone	Confirmed	4 683	978
	Probable	79	174
	Suspected	824	35
	All	5 586	1 187
Mali	Confirmed	3	3
	Probable	1	–
	Suspected	0	–
	All	4	3
Spain	Confirmed	1	–
	Probable	–	–
	Suspected	–	–
	All	1	0
USA	Confirmed	4	1
	Probable	–	–
	Suspected	–	–
	All	4	1
Total			

–: N/A

## 2.1. Transmission of Ebola virus

It is considered that fruit bats of the Pteropodidae family are natural hosts of Ebola virus. Ebola is introduced into the human population through close contact with the blood, organs, secretions or other bodily fluids of Ebola infected animals such as gorillas, chimpanzees, monkeys and fruit bats[10].

Generally, human to human transmission occurs due to direct contact with infectious body fluids or blood. The virus gets entered into body through the gastrointestinal tract, conjunctiva or breaks in the skin the body. The maximum virus concentrations are found in blood while lower levels are present in urine and throat washings[14,15].

## 2.2. Symptoms of Ebola virus disease

It takes about 2–21 days from viral infection to onset of symptoms (incubation period).

Initial symptoms include sudden onset of fever fatigue, headache muscle pain and sore throat which is followed by vomiting, rash, diarrhea, symptoms of impaired liver and kidney function (Figure 3). In some cases, both external and internal bleeding *e.g.* oozing from the gums, blood in the stools[10,16].

## 3. Tobacco plant: a key to Ebola

In the world of health and medicine, tobacco is usually considered as a threat to human health, but recently scientists are looking to tobacco plant as a vehicle to synthesize proteins *i.e.* antibodies (Figure 4).

### 3.1. History of tobacco plant in medicine

Tobacco has been used by native cultures since several years before the time Christopher Columbus reached America in 1492. The popularity of tobacco was possibly because of its dual nature *i.e.* small quantity of tobacco produces a mild stimulating effect on the user where as a large amounts can cause hallucinations or even death. So that's why it is an integral part of various cultures and plays a key role in many shamanistic traditions. In pre-Columbian North America, various civilizations preferred it for smoking and for many other purposes *e.g.*, healing ailments (especially to cure earaches, cuts and burns, snake bites) respiratory diseases, urinary ailments, nervous ailments, fever, convulsions and skin diseases[9,17-23]. Brief description of tobacco usage history in medicine is presented in Table 2.

### 3.2. The pharmaceutical benefits of tobacco plants

Nowadays, variety of vaccines are prepared from tobacco plants

**Table 2**

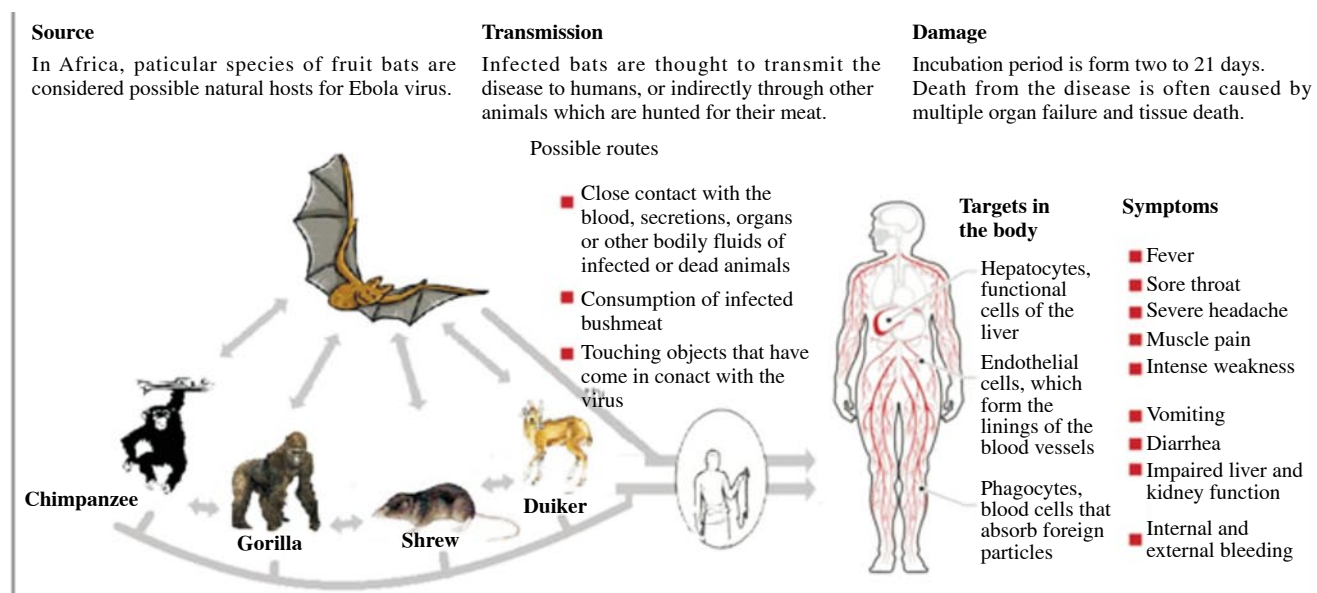
Historical use of tobacco in medicine

Time period	Uses	Citation
Pre-Columbian America	Tobacco, mixed with lime or chalk is used by Native American populations as toothpaste to whiten the teeth.	[17]
Early use in Europe	In 1529, a Spanish missionary priest, Bernadino de Sahagun, recorded that breathing the odour of fresh green leaves of the plant, relieved persistent headaches. For colds and catarrh, green or powdered leaves should be rubbed inside the mouth. Goitre could be cured by cutting out the root of the lesion and placing on it crushed tobacco plant hot and mixed with salt, on the same spot.	[18]
	In 1612 Vaughan declared that 'tobacco well dried, and taken in a cleane Pipe fasting cure the megrim, the toothache, obstructions proceeding of cold and helpeth the fits of the mother', warned that it could do much harm when abused.	[19]
The 19th century	In the 17th century, John Wesley's Primitive Physick, first published in 1747, recommended tobacco for earache and for falling sickness, and for piles. Such advice continued as late as the edition of 1847.	[20]
	Subsequently Stewart find out that if tobacco is administered externally it cure bites of poisonous reptiles and insects; hysteria; pain, neuralgia; laryngeal spasm; gout; growth of hair; tetanus; ringworm; rodent ulcer; ulcers; wounds; respiratory stimulant and when administered by mouth can cure strangulated hernia (smoke by mouth); malaria or intermittent fever; dislodging obstructive material from oesophagus by inducing vomiting when tobacco is administered by rectum it cure constipation and haemorrhoidal bleeding.	
The 20th century and after	Later in the 20th century it is observed that in at least three case-control studies, the relative risk of Parkinson's disease was lower in smokers than in non-smokers, though other factors could be operating to produce this apparent effect so tobacco could cure disease affecting the brain and nervous system.	[21]

**Table 3**

Some examples of biopharmaceuticals produced in tobacco plant[30].

Protein	Company/Organization	Indication/Application	Development stage
Animal vaccine	USA, Dow AgroSciences	Newcastle disease in chicken	Approved by USDA2/2006
Monoclonal antibody	USA, Planet Biotechnology	Prophylaxis of caries	Phase 2
Antibody, cancer vaccine	USA, Large Scale Biology	Non-Hodgkin Lymphoma	Phase 2
Antibody	USA, Planet Biotechnology	Cold caused by Rhinoviruses	Phase 2



**Figure 3.** Transmission and damage of Ebola virus[16].

by pharmaceutical companies e.g. a vaccine for non-Hodgkin's lymphoma[21] and Newcastle disease virus (NDV) vaccine[22]. There are also many reports of plants made antibodies i.e. tumor targeting or cancer treatment[23,24], prevention of tooth decay[25] and preventing sexually transmitted disease[26].

Various other plant species such as potato plants are also utilized for production of antibodies, however contribution of *Nicotiana* species appears to be maximum i.e. 74.4% total [*Nicotiana tabacum* 58.1% or *Nicotiana benthamiana* (*N. benthamiana*) 16.3%] (Table 3)[27].

**3.3. Pharming in tobacco plant**

Pharming in plants has emerged as a niche technology for the production of pharmaceutical products designated for chronic and infectious diseases. Through genetic engineering, genes that code for useful pharmaceuticals are inserted into host plants that thus create a genetically modified organism (GMO)[28].

Tobacco plant, because of its high biomass yields and rapid scalability is by far the most desirable choice in many studies on plant-based antibodies and proteins. Additionally, it is

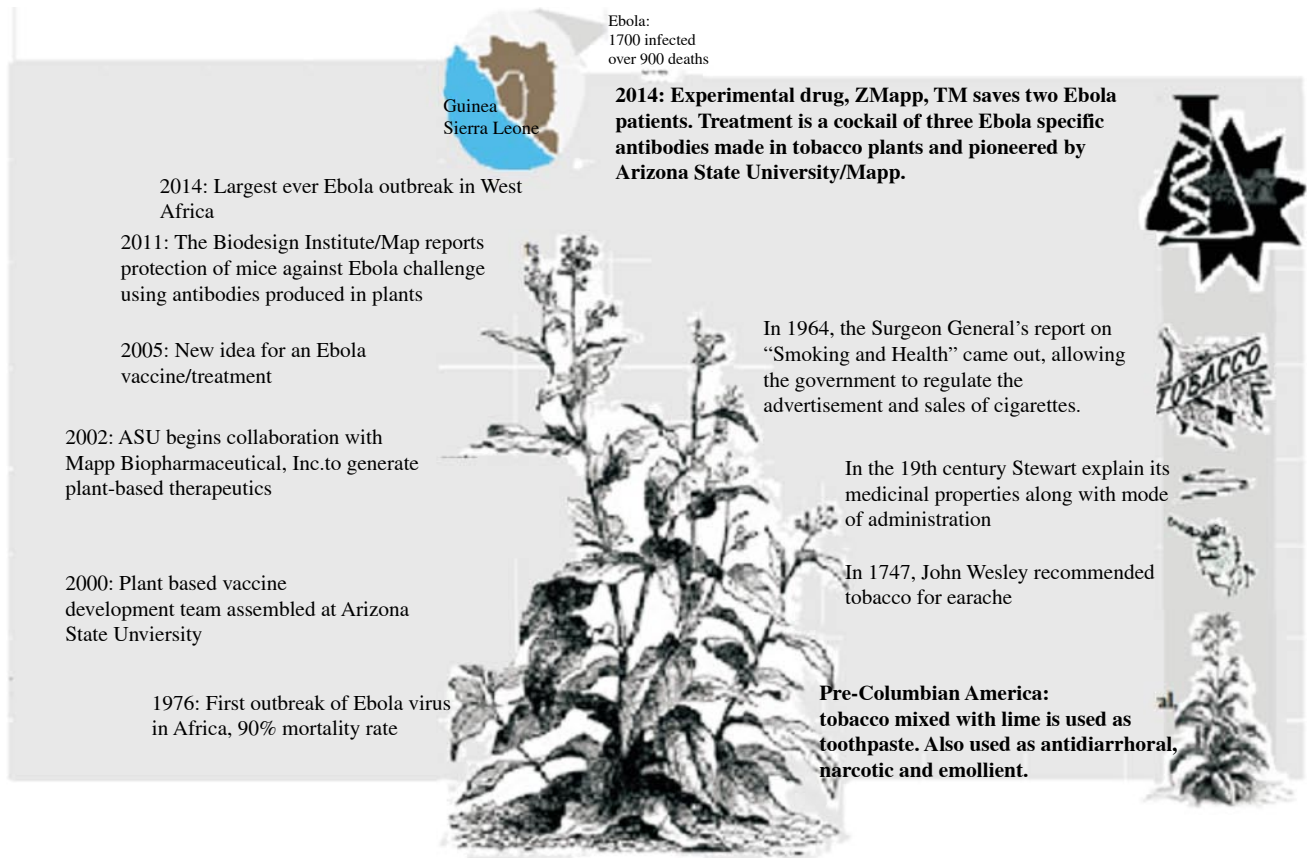


Figure 4. Tobacco plant history and Ebola vaccine.

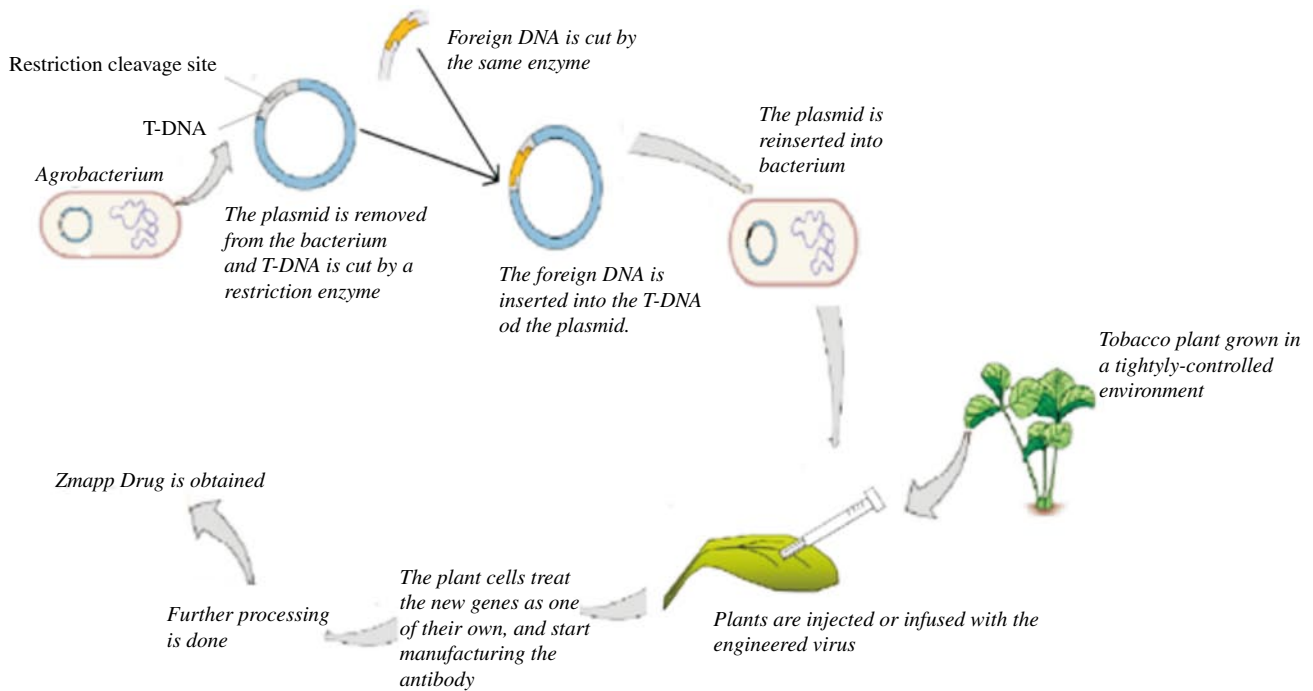


Figure 5. Production of Zmapp through tobacco plant.

not a food crop, and so carries an abridged risk of transgenic material contaminating food and feed supplies. A wide array of pharmaceuticals produced by tobacco plant based pharming is now in the pipeline for commercialization to treat diseases such as cystic fibrosis and non-Hodgkin's lymphoma (Table 2)[29].

### 3.4. ZMapp drug and Ebola

ZMapp drug is manufactured through a bioproduction process in the tobacco plant *N. benthamiana* by Kentucky BioProcessing. It is combination of three monoclonal antibodies *i.e.* c13C6 from



a formerly existing antibody cocktail called “MB-003” and two chimeric mAbs from a different antibody cocktail called ZMab, c2G4 and c4G7 that have been produced by genetic engineering[20]. ZMapp cocktail contains neutralizing antibodies like intravenous immunoglobulin therapy that offer inert immunity to the virus by specifically and directly reacting with it in a “lock and key” fashion[31]. ZMapp, had verified efficacy against Ebola infection in nonhuman primates and also successfully treat two American medical workers infected with Ebola virus disease[32].

The ZMapp three-antibody cocktail binds to the Ebola virus to deactivate it and provides simulated immune response against sugar-tagged proteins on the outside of the Ebolavirus. Ready-made antibodies produced in the laboratory to latch onto specific parts of an infectious agent and then are inserted into body which results in an immediate immune response. Therefore ZMapp is different from a vaccine that might require weeks for the person to make their own antibodies against the virus[32].

### 3.5. Production of Zmapp through tobacco plant

To produce the drug, tobacco plants are not genetically altered or modified in any way. First plant virus (*Agrobacterium*) is combined with the genes of the Ebola antibodies needed for the drug as a result the virus starts producing a lot of that antibody protein. Then *N. benthamiana* seeds are planted and allowed to grow in a tightly-controlled facility that maintains optimal levels of light, temperature and humidity for the plant for 6 weeks, so that their leaves are large enough to serve as a factory for making antibodies[33].

Then plants are injected or infused with the engineered virus (viral vector) encoding Ebola antibodies. The plant cells treat the new genes as one of their own, and start manufacturing the antibody[12]. Plants are then allowed to grow even more then harvested and processed in order to separate the antibodies from the rest of the plant then purified, tested for potency and then made into the drug known as ZMapp[34].

Plant based manufactured drugs are less expensive and represents a new approach of producing drugs that can lessen the time it takes to bring serious medications. In order to produce human antibodies, tobacco plant takes less time than it takes to genetically engineer a mouse or other rodent. It is also easy to contain and manufacture in controlled environments. As compare to mammalian cell based products, each tobacco plant can produce enough antibodies for dozens of doses of a pharmaceutical.

### 3.6. Experimental studies on ZMapp drug

The development of ZMapp is the outcome of cooperation

between three pharmaceutical companies in the US and Canada, the Public Health Agency in Canada and the US Government[34]. So far ZMapp (a new treatment for Ebola virus) has not been tested in humans but has been tested on animals.

A recently published study demonstrated that when the treatment is instigated up to 5 days post-challenge, ZMapp is able to rescue 100% of rhesus macaques. It is also observed that advanced disease, as indicated by mucosal haemorrhages, elevated liver enzymes and generalized petechia could be upturned, leading to full recovery. Enzyme-linked immunosorbent assay and neutralizing antibody assays signify that ZMapp is cross-reactive with the Guinean variant of Ebola[35], and another experimental study proved that ZMapp completely protected monkeys against a lethal dose of Ebola virus. Unlike other post-infection therapies, the ZMapp treatment of Ebola virus works even at advanced stages of the disease[34].

During the current outbreak of Ebola, ZMapp has been given to a small number of Ebola patients and a British nurse was discharged after a complete recovery in early September[36].

## 4. Conclusion

Ebola virus disease is not just a problem for Western Africa but it is a serious global issue. The success of ZMapp Ebola therapy indicates a new age of drug manufacturing involving tobacco plants. In addition to ZMapp, production of various other vaccines has been made with the plants, and the possibilities are endless. But research funding are required all over the world in order to explore the unexploited potential of plants in pharmaceuticals, because it is expected that plants will continue to become efficacious, safe and economical platforms for extensive production of vaccine and other biopharmaceutical proteins against a large range of infectious diseases. Plants based are easily administered to people who live in distant areas of the developing world because they do not require refrigeration. That’s why they are possible alternative to conventional vaccines, and can address the space exist in the infrastructure accessible to improve global public health.

### Conflict of interest statement

We declare that we have no conflict of interest.

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