Section 4

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Pharmacology

Zedupex, an Anti-herpes Herbal Medicine for Management of Human Herpes

Kenya

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Summary

The KEMRI/JICA Infectious Diseases Research Project (2001-2006) was a collaborative study between the Kenya Medical Research Institute (KEMRI) and the Japan International Corporation Agency (JICA), the main purpose of which was to improve and enhance research and production capacity, human resources and human/information networks at KEMRI in collaboration with other institutions in Kenya and the region. The aim of the project was to strengthen effective control of targeted diseases (HIV/AIDS, viral hepatitis and opportunistic infections) through the identification of new management therapies and tools. A sub-project carried out by the Plant Drug Research Group (PDRG) was to produce effective, safe and chemically well-characterized herbal-based products that could be developed for use against opportunistic viral infections caused by the herpes simplex virus. Herpes is caused by two sub-types of the human herpes simplex virus (HSV-1 and HSV-2) which share a 50% gene sequence homology. The viruses can initiate and establish recurrent orofacial lesions and are clinically indistinguishable from initial episodes of genital herpes.

Studies on HSV seroprevalence reveal rates of up to 80% in Africa. Increased prevalence rates have been attributed to immunosuppression, mainly due to HIV/AIDS infection, when HSV is a major opportunistic pathogen. Even though the chemotherapy of HSV infections has improved tremendously over the years, the management of these infections in Africa has been hampered by the high costs of the drugs. Identification of local therapies for management of the infection is therefore paramount given that over 60% of the population depends on herbal medicine for primary health care.

Background and Justification

Herpes is a major health concern around the world and in Africa in particular. In sub-Saharan Africa, seroprevalence of herpes simplex virus type 2 (HSV-2) is among the highest in the world, and records of 50-80% have been registered in population-based studies (Looker *et al.*, 2015). In Kenya, according to the 2009 Kenya AIDS indicator survey report that provided the first report on herpes prevalence in the country, over 6 million adults were HSV-2-infected (Mugo *et al.*, 2009). In the region, HSV is the leading cause of genital ulcer disease (GUD), an infection that enhances the infectiousness of HIV-positive subjects and the susceptibility of HIV-negative subjects to HIV infection. The widespread nature of herpes infection in sub-Saharan Africa is attributable to malnutrition on one hand and immunosuppression due to HIV/ AIDS on the other, compounding factors which contribute to the manifestation of the opportunistic nature of the HSV.

Despite the strengths of the epidemiological evidence of herpes presence in sub-Saharan Africa, treatment is not readily available even though great achievements have been realized in the development of new therapeutic agents for its management. In instances where these drugs are available, they are too expensive and therefore unaffordable by most people in the region. For this reason, there is a need for the development of affordable therapeutic agents to address the problem in Africa. Another reason is the fact that most widely used drugs for prophylaxis and treatment of HSV infection are acyclovir-based but unfortunately, they have high toxicities and long-term therapy associated with their use leads to the development of clinically resistant viral strains.

The KEMRI/JICA Infectious Diseases Research Project (2001-2006) was a collaborative study between Kenya Medical Research Institute (KEMRI) and the Japan International Corporation Agency (JICA), aimed at strengthening medical research. The main goals of the project were to identify ways to strengthen effective control of problematic diseases, such as HSV, in the region through identification of new management therapies and tools. The Plant Drug Research Group (PDRG) at KEMRI thus aimed at producing effective, safe and chemically well-characterized herbal-based products that could be developed for use against opportunistic viral infections caused by herpes causing viruses.

Description

Herpes is a virus infection of the skin and mucosa caused by the virus, human herpes simplex virus (HSV), sub-type 1 (HSV-1) or sub-type 2 (HSV-2) and manifests as cutaneous skin lesions of the genitals (genital herpes) and/or oral facial infections (oral labial herpes) (Fig. 1). The two sub-types of the virus are among the most common causes of viral infections of humans worldwide, resulting in a spectrum of illnesses ranging from asymptomatic to life-threatening disease. These two viruses share a 50% gene sequence homology and can initiate and establish recurrent orofacial lesions and similarly cause clinically indistinguishable first episodes of genital herpes. Herpes can be transmitted through sexual contact, kissing and skin-to-skin contacts.

Oral labial herpes ***Genital herpes***

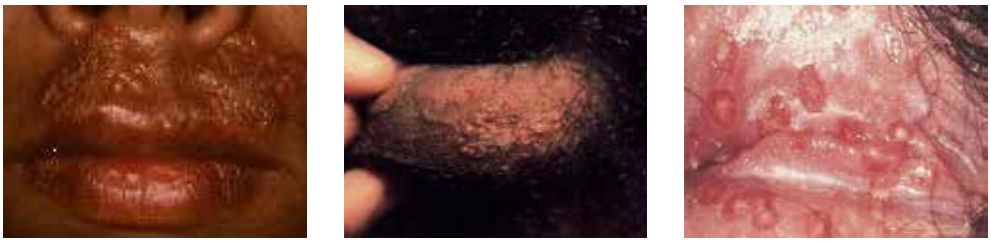


Figure 1: Symptoms of oral labial herpes (left), and genital herpes in the male (centre) and female (right).

The association of herpes infection with human immunodeficiency virus type HIV/AIDS acquisition and manifestation continues to provide a worrying challenge. Increasing evidence demonstrates a substantial link between the epidemics of sexually transmitted HIV-1 and genital herpes in developing countries, which is a matter of great public health concern. Over the years, data from Africa, Asia and the Americas have highlighted the parallel and intersecting epidemics of HIV-1 and HSV-2, with a growing understanding about the impact of genital HSV infection on increased risk of HIV-1 acquisition. Another compounding factor of HSV infection is its role as a major cause of genital ulcer disease (GUD) in both developed and developing countries. A report of the International Herpes Management Forum (IHMF) indicates that HSV infection has overtaken bacterial sexually transmitted disease (STD) infection as the most common cause of GUD disease worldwide. GUD enhances the infectiousness of HIV-positive subjects and the susceptibility of HIV-negative subjects to HIV infection.

Zedupex is prepared from a medicinal plant which grows in its natural habitat in the Kenyan forest. A voucher specimen (No. 0077: Mungai, Rukunga and Tolo) is deposited at the East African Herbarium, Nairobi, Kenya. The bark of the plant is dried and ground into fine powder from which a hot water extract is prepared and lyophilized. The lyophilized powder is then evaluated for the presence of an active compound as a standardization measure before formulation (Fig. 2).

Phytochemical screening of the raw material of Zedupex has shown the presence of several classes of compounds with potential pharmaceutical activity, including alkaloids, phenolics, flavonoids, anthraquinones, terpenoids, steroids and saponins.

Product formulation

Peeled bark of the medicinal plant being processed for grinding.

Ground bark ready for phytochemical screening/weighing and packing or extraction/ lyophilisation/cream formulation.

An aqueous extract of the plant being lyophilized in 200ml aliquots.

Formulated Zedupex cream prepared from the lyophilized extract.

Figure 2: Procedure for preparing standardized formulation of Zedupex from the bark of a medicinal plant native to Kenya.

Product standardization (Quality assurance and control)

The presence of a lead compound that has been isolated from the extract of the medicinal plant from which Zedupex products are prepared is always determined. The compound is the biological marker for activity and its presence in any freshly collected and processed material is determined as a quality control (QC) measure. Quality assurance (QA) is verified at each major stage in the production process for each batch. At the end of the production line, the finalized product, Zedupex powder or cream, is evaluated for anti-HSV activity before the batch is released (Fig. 3).

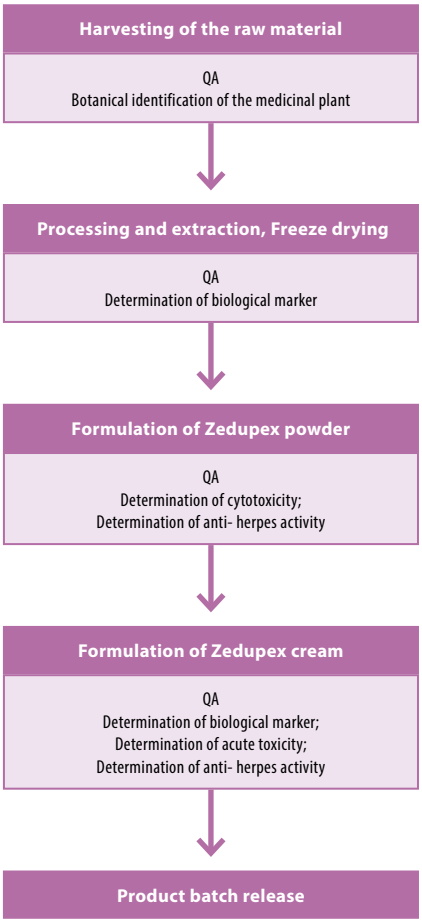


Figure 3: Quality assurance (QA) at major production points of Zedupex.

The product is presented in two forms: powder and cream. Zedupex powder is a preparation of a finely ground dried raw material of the medicinal plant. For herpes management, one single oral administration for an adult of an average weight of 70kg consists of boiling water and adding 1 teaspoonful of the powder (1.0 g approx.) to a cup of hot water (70 mls approx.) and stirring, letting it settle for 10 minutes, sieving and drinking. A child, of age above 10 years, can take half this dosage. The extract can be taken three times a day for a week or until the herpes lesions clear completely.

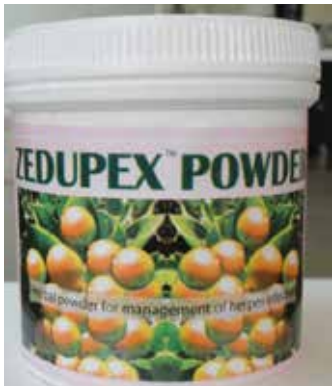


Figure 4: Commercially availabe packs of Zedupex powder (left) and cream (right).

Zedupex cream is a formulation of an extracted and freeze dried portion of the medicinal plant. The cream has no chemical additives besides a preservative and its brown colour and aromatic smell originate from its raw materials. The cream contains a 10% freeze dried portion (10mg w/w) in an aqueous cream base. The cream is for topical use only to be applied evenly on the affected area three times daily. The treatment should be continued for at least one week, or until the herpes lesions clear completely.

The efficacy of Zedupex was confirmed in a series of laboratory tests.

Anti-herpes activity of Zedupex on mammalian cell lines on wild type and acyclovir resistant strains of herpes simplex virus

Table 1 shows the effective concentrations of Zedupex to cause 50% viral death (EC_{50}) in vitro, as well as the cell cytotoxic concentration of the product which causes 50% cell death (CC_{50}) in the absence of viral infection. It is important to note that the CC_{50} (480 µg/ml) is well above the EC_{50} (15.1 µg/ml) meaning that the formulation has a high selectivity index and is therefore very safe and that it works well against both sensitive and resistant strains of HSV.

Table 1: Effective concentrations of Zedupex causing 50% viral death (EC_{50}) in vitro against both sensitive and resistant strains of HSV; and the cell cytotoxic concentration of the product causing 50% cell death (CC_{50}) in the absence of viral infection. (The results are means of three independent experiments).

Formulation/ Drug	EC_{50} [a] ± S.D (µg/ml)				CC_{50} [b] (µg/ml)
	HSV-1	HSV-2	HSV-1 AP^r	HSV-1 TK^-	
Zedupex	15.1±0.57	6.9±1.27	8.1±1.56	11.1±5.66	480.00
Acyclovir (Zovirax)	0.91±0.46	0.87±0.44	>5.0	>5.0	>100

[a] Effective concentration for 50% plaque reduction;

[b] Cytotoxic concentration causing 50% cell lysis.

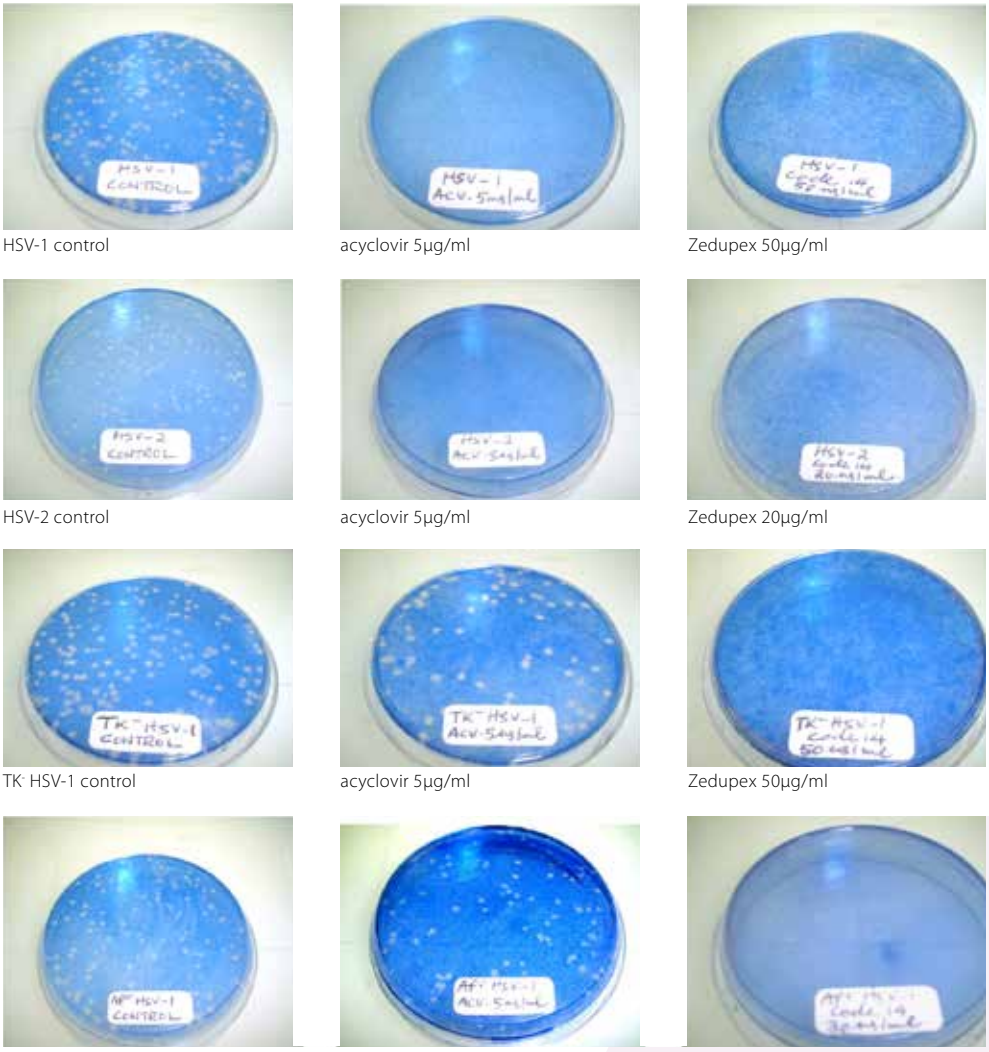
HSV-1 Herpes simplex virus type 1;

HSV-2 Herpes simplex virus type 2;

HSV-1 AP^r Acyclovir resistant strain of HSV type 1;

HSV-1 TK^- Another type of acyclovir resistant strain of HSV type 1.

Figure 5 shows that 50µg/ml Zedupex completely stops the replication of the virus in both sensitive (HSV-1 and HSV-2) strains and resistant (HSV-1 and AP^r HSV-1) strains, whereas the commonly-used acyclovir has little effect on the resistant strains as evidenced by the persistence of the white viral plaques.



HSV-1 control | acyclovir 5µg/ml | Zedupex 50µg/ml

HSV-2 control | acyclovir 5µg/ml | Zedupex 20µg/ml

TK$^-$ HSV-1 control | acyclovir 5µg/ml | Zedupex 50µg/ml

AP^r HSV-1 control | acyclovir 5µg/ml | Zedupex 30µg/ml

Figure 5: Effects of acyclovir and Zedupex against sensitive and resistant strains of HSV. White spots are viral plaques (areas infected with the virus, most evident in no-treatment controls, left column).

Following these successful *in vitro* trials, we moved on to the animal experiments with mice and guinea pigs once our protocols had been approved by KEMRI's Animal Care and Use Committee (ACUC). Again, these experiments were successful in treating HSV and showed no ill effects.

The plant powder was then formulated as either a cream and pre-clinically evaluated for herpes infection and intended for use in herpes management in humans. As herbal products, their ethnomedical use in crude form for human disease management over the years established their safety and this has now been confirmed experimentally. An evaluation of efficacy in controlled pre-clinical settings provides indicators of their potential as antiviral agents and an evidence base for clinical evaluation. Proper clinical evaluations have yet to be done. However, given the magnitude of herpes infection in Kenya, the formulated products have been made available to registered traditional health practitioners (THPs) for prescription in their clinics. This use is being monitored through observational studies by qualified clinical doctors. Reports so far indicate a positive response in management of herpes infection. Data on observational studies is still limited and will be made available once ethical clearance has been received.

Partnerships

During this project, KEMRI researchers collaborated with partners from the National Commission for Science, Technology and Innovation (NACOSTI), Nairobi, and JICA.

In addition, collaboration with Kenyan THPs registered by the Ministry of Culture and Social Services, Gender and Sport was critical.

Impact

The study has demonstrated the important role herbal medicine could play in health management if quality control, efficacy and safety of herbal preparations are established and products formulated in suitable dosage forms for ease of use. The formulated herbal medicines could be relatively cheaper than conventional therapies since the raw materials could be sourced locally and their preparation would not entail complex pharmaceutical processing. Importantly, the documented scientific information on them would enhance the evidence base for their integration into mainstream healthcare systems in Africa and beyond.

In October 2012, the team won a prize for the best innovative researched natural product at the Nairobi International Trade Fair, Kenya, awarded by His Excellency the President of the Republic of Kenya.

Replicability

As explained above, herpes is a burden not only in Africa, but across much of the rest of the world. Once fully researched with positive results, Zedupex would contribute significantly in lessening the suffering experienced following infection. As much as it cannot be claimed to be a cure for herpes, indications have shown that the frequency of clinical presentation of infection following a latent HSV phase is remarkably reduced with Zedupex use. As with any other health product, the benefits of Zedupex could therefore be experienced in any region of the world.

KEMRI has thus applied for a patent for: "A novel anti-viral plant extract". In the meantime, Zedupex has been listed by the Pharmacy and Poisons Board of Kenya.

Presentation at workshops, seminars and scientific conferences, both locally and internationally, will provide evidence of the scientific merit of the product as an antiviral agent and propel its integration into healthcare management systems.

Lessons Learned

A major obstacle faced in the early stages of research was the sourcing of the raw material. Before the current site was identified, the raw material was obtained from a range of geographical localities which gave rise to difficulties in standardization. This was because the different localities provided different qualities of raw material. Presently, the project is planning to develop a herbal garden of the medicinal plant for large-scale production to guarantee supplies of the raw material.

Future Plans

There are plans to mount proper clinical studies of the Zedupex product. A study protocol has been developed for Phase 1 and 2 clinical trials at the Centre for Clinical Research (CCR) of KEMRI. However, lack of funds has delayed the onset of the study. Project funding is being sourced from the Government of Kenya and possible collaborators.

Once clinical trials are conducted and concluded, a pharmaceutical company will be approached for partnership in product production and sales. Discussions and agreements will be guided by KEMRI's Intellectual Property Rights (IPR) policy in tandem with regulations of the partnering entity.

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