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REVIEW PAPER

Prevention of AIDS and Sexually-transmitted Infections

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ABSTRACT

Abnormal vaginal discharge due to reproductive tract infections (RTIs) is widely prevalent in the country. According to WHO, over 300 million new cases of sexually-transmitted infections (excluding HIV) occur each year. In addition to these, HIV infection is spreading rapidly in the country with over 3.7 million sero-positive cases (from zero) within 15 years. The predominant mode of transmission of HIV is by heterosexual route. The multidrug regime for treatment is expensive (about $10, 000 per year) which is beyond the reach of most of the people. No viable vaccine preventing AIDS infection is in sight. Under these circumstances, safe sex is the best recourse, which demands consistent and proper use of condoms. This does not take place to the extent necessary for preventing the transmission of HIV and other sexually-transmitted infections. A polyherbal tablet for intravaginal use by women has been developed, and a polyherbal cream, for use by both men and women, which has a wide spectrum antimicrobial action. Amongst others, these inhibit the growth of *Neisseria gonorrhea* (including strains resistant to penicillin), normal and multidrug-resistant isolates of urinary tract *E. coli*, *Candida albicans*, *Candida krusei* and *Candida tropicalis*. Applied intravaginally, these prevent the transmission of *Herpes simplex* 2 and *Chlamydia trachomatis* in progestin-sensitised mice. Studies conducted at the Institut Pasteur, Paris and Conrad Norfolk, USA, have demonstrated high virucidal action of these formulations against HIV1. Both formulations have undergone phase I clinical trials in five major centres in India and abroad, which have indicated the complete safety of these products without any local or systemic side effects. The Drug Controller of India and the Institutional Ethics Committees have approved phase II clinical trials. The first of these trials was conducted in 88 women with abnormal vaginal discharge due to genital pathogens at the Postgraduate Institute of Medical Education and Research, Chandigarh. Every woman, who used the *praneem* polyherbals, once every night for seven days, experienced relief clinically, irrespective of the nature of the causative pathogen.

Keywords: AIDS, sexually-transmitted infections, polyherbal formulations, reproductive tract infections, spermicides, microbicides

1. INTRODUCTION

Since late, increasing attention is being given to the reproductive health of women. Bang[1], *et al.* reported that nearly 50 per cent of women in rural Maharashtra suffered from abnormal vaginal discharge due to reproductive tract infections (RTIs). An independent study of Indian Council of Medical Research[2] revealed that 27 per cent to 30 per cent of women attending ante-natal clinics in city hospitals had RTIs. The Population Foundation of India noted that nearly 80 per cent of women in slums have abnormal vaginal discharge due to RTIs. The incidence of RTIs is thus high in the country. Women do not approach the doctors for these problems, due to privacy and cultural factors. Many feel that these are natural consequence of their married life. The causative organisms vary, ranging from (aerobic and anaerobic) microbes to fungi, protozoa and viruses.

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There is also an apparent epidemic of sexually-transmitted infections (STIs). WHO reported[3] 333 million new cases of STIs in the world in 1995, amongst which 62 million were of *Neisseria gonorrhea*. These figures do not include the AIDS pandemic raging in the country. The AIDS virus is of foreign origin and first noticed in India in 1986 (leaving aside a few cases of AIDS of Indians who had contracted the disease overseas). Initially, the epicentres of infection were confined to two port cities, Chennai and Mumbai. To these was added a foyer of intravenous drug users in Manipur and in areas bordering Mynamar. Over the past 15 years, the HIV infection is no longer restricted to the epicentres. The entire states of Tamil Nadu, Maharashtra and Andhra Pradesh are now endemic areas. In the northeast, the infection has creeped to West Bengal, and the entire trucks route from Mumbai to Amritsar is today infected with HIV which is passed on to innocent wives in villages. In Maharashtra, sero-positivity of monogamous married women coming for delivery to hospitals is now 0.4 per cent to 2.7 per cent. These represent in a way the general population and not the professional sex workers. We are sitting on an iceberg and many steps must be taken to contain the HIV pandemic. Awareness and education for safe sex is being spread by various agencies– although more needs to be done. The main reliance for safe sex is on the use of condoms. Condoms are largely male controlled, who do not use these regularly. A highly useful introduction will be the vaginal microbicides under full control of women. Development of two polyherbal formulations intended for this purpose has been briefly reviewed here.

2. SPERMICIDES-CUM-MICROBICIDES

Spermicides-cum-microbicides are available in the form of suppositories (pessaries), foaming tablets, creams, or sponges soaked with solutions of compounds. The most commonly used compound is nonoxynol-9, a powerful detergent with high spermicidal and microbicidal properties. Another compound used in some preparations is benzyl alkonium chloride. These compounds, while effective as spermicides, are cytotoxic and their repeated use causes inflammation of the vaginal mucosa and damage to the vaginal epithelium[4] with the result that viral uptake is enhanced rather than the killing of the virus. Trials conducted in Kenya on professional sex workers employing nonoxynol-based spermicides gave the surprising results of an increased uptake of HIV infection[5]. It was postulated that reduction of the concentration of nonoxynol-9 by slow release of formulations and coverage of the vaginal epithelium by bio-adhesive materials incorporated in the microbicide formulation may overcome the noxious effects of nonoxynol-9. Advantage-S, an improved gel, incorporating these features, which is marketed by a New York-based company, underwent phase III clinical trials under the auspices of UN-AIDS. Unfortunately, the results of this carefully planned trial showed that this gel was not effective in preventing the transmission of HIV infection by the vaginal route. The spermicide marketed in France with benzyl alkonium chloride as base has met with a similar fate. It failed to prevent the transmission of simian immunodeficiency virus (SIV) in monkeys by the vaginal route. At present, there is thus no spermicide-cum-microbicide of proven efficacy available to women for protection against AIDS and sexually-transmitted infections.

The contraceptive efficacy of the available spermicides used as a single method for preventing pregnancy is reported to be around 80 per cent, which is lower than that of steroid pills or intrauterine devices. In part, this low efficacy may be ascribable to improper use of the products . However, there may be scope for further potentiating the spermicidal properties.

3. STRATEGY ADOPTED

It was concluded that in a vaginal-use product, a higher spermicidal action is required without the use of nonoxynol-9 or benzyl alkonium chloride which damage the vaginal lining. Instead of synthetic compounds, it was considered to use extracts of plants, employed traditionally for various purposes, which are devoid of any side effects, and may have spermicidal properties.

These included *neem* (*Azardirachta indica*), *Haldi* (*Curcumin longa*) and others. These plants

have been used topically on injuries without causing irritation or any undesirable side effects. These are even ingested without any stated systemic side effect. Thus small uptake of such compounds through skin, if it were to occur, would not be harmful to health. The author determined whether *neem* seed and/or leaf extracts have spermicidal properties on human sperm as tested by Sander Cramer slide test. This was the case[6]. However, concentrations required to kill 100 per cent sperms in 20 s were high. Riar[7], *et al.* attempted to concentrate the spermicidal properties in the *neem* oil and identified a fraction, nim-76, which, according to them, contained most of the spermicidal activity. The DRDO laboratories decided to make *neem* oil and nim-76 as the basis of a method for family planning. Credit must be given to the pioneering work of Dr Riar, and to Dr Selvamurthy and his colleagues, for further development of this lead.

We adopted a different strategy, in which one looked for odourless and colourless extracts from *neem* (to avoid staining of undergarments). It was difficult to remove pungent odour of *neem* oil without loss of spermicidal properties. So, extracts from *neem* leaves which were further purified to obtain odourless, colourless fraction (*praneem*) containing spermicidal activity. Fingerprint profiles of this fraction were developed, so that batches of reproducible properties could be obtained[8,9]. To potentiate the spermicidal properties, the author employed more than one plant-derived compounds endowed with spermicidal properties. Extracts of soapnut (*Sappindus mukerosi*) have been used for centuries for washing of hair, cleaning of delicate woollen garments and precious carpets. These were active at fairly low concentrations. To add fragrance to the product, *Mentha citrata* oil, quality controlled as per gas-liquid chromatography (GLC) profiles and bioactivity was chosen. Interestingly, the combination of these three were synergistic and enhanced spermicidal properties by eight-to-twelve-fold.

4. WIDE SPECTRUM ANTIMICROBIAL ACTION

Neem is a miracle tree[10]. It makes about 100 tri, tetra and penta terpenoids of fairly complicated structures. The chemistry of many such compounds has been elucidated by the works of Siddiqui[11], Govindachari[12], Schmutterer[13] and others. *Neem* is reported to have antibacterial[14], antifungal, antiviral[15], antinematode and antilarval properties[16]. The mechanism by which such action is exercised has been studied for *Azadirachtin*. It is an elegant two-step process[17]. On one side, *neem* constituents exercise anti-feedant action on insects, which are discouraged to feed on *neem* leaves or on vegetation sprinkled with *neem* extracts. On the other side, *Azadirachtin* blocks the development of larva, acting as an antagonist to the action of ecdysone and juvenile hormone. What an ecologically sound and elegant way to control insects! First they do not feed, and if they have passed that stage, do not grow into new insects. The mechanism of action of *neem* components on bacteria or viruses is not fully known. *Neem* extracts do inhibit the NFKb-induced replication of HIV in a model experimental system investigated by the author in collaboration with Dr D.K. Biswas and Prof A.B. Pardee, Harvard Medical School, some years back. In other studies, it was observed that *neem* seed extracts activate macrophages[16] and stimulate cell-mediated immune responses[18]. In high concentrations, given orally, *neem* seed extracts cause abortion[19] and the mechanism is by the release of inflammatory cytokines, such as gamma interferon and TNFα[20], characteristic of Th_1 type of immune response. Saponins should have cell membrane labilising action. *Praneem* polyherbal formulations have three components, purified *neem* leaf extracts (*praneem*), saponins from *Sappindus mukerosi* and *Mentha citrata* oil. These are dispensed as a pessary or tablet and as cream.

In collaborative studies with Dr Ashok Rattan and Dr Uma Bannerjee at the All India Institute of Medical Sciences (AIIMS), the growth inhibitory action of the *praneem* polyherbal formulations was observed on *Neisseria gonorrhea* (including penicillin-resistant strains), urinary tract *E.coli* (including multidrug-resistant strains), *Candida albicans*, *Candida Krusei* and *Candida tropicalis*[21]. In another study carried out in collaboration with Dr Kevin Whalley and Sharon Achilles of Johns Hopkins University, pre-application of *praneem*

in vagina of progestin-sensitised mice 20 s before inoculation of *Herpes simplex* 2 prevented the development of lesions due to *Herpes*, and no cultivable virus was present in the vagina on days 4 and 8 after infection. In a similar *in vivo* experimental model, *praneem* was highly effective in blocking the transmission of *Chlamydia trachomatis* infections[21].

The high virucidal effect of *praneem* polyherbal on HIV 1 has been investigated in two laboratories: at the Institut Pasteur, Paris and Conrad Norfolks, USA. The systems for study differed. However, both studies demonstrated a high virucidal action of *praneem* polyherbal on virus causing AIDS[21].

5. CLINICAL EVALUATION

Pre-clinical toxicology studies were conducted in rats and monkeys, which showed the safety of *praneem* polyherbal[22]. Draize test on braided skin of rabbits and cumulative studies on human skin indicated the lack of irritability of the formulation[23]. After due permission of the Drugs Controller of India and Institutional Ethics Committees, phase I clinical trials were conducted with the *praneem* polyherbal pessary in 23 women at three centres, namely Postgraduate Institute of Medical Education and Research, Chandigarh (PGIMER), Safdarjung Hospital, New Delhi and Kamla Nehru Hospital, Allahabad. These consisted of local reactions, if any, effect on vaginal cytology, and on systemic indices of hematology, blood chemistry and organ functions after seven consecutive days of intravaginal application of *praneem* polyherbal pessary. Phase I clinical studies with the *praneem* cream were also carried out in Brazil. These studies showed the safety and lack of toxicity of *praneem* polyherbal formulations.

Phase II efficacy studies were undertaken in women suffering from abnormal vaginal discharge due to RTIs. These studies had the clearance of the Drugs Controller of India and the Institutional Ethics Committees. The Population Foundation of India funded the study at the PGIMER, Chandigarh. The efficacy was studied in 88 women enrolled on the basis of thorough clinical and microbiological examinations. Women were prescribed to insert *praneem* polyherbal pessary/tablet high in the vagina with washed fingers every night for seven days and report back to the clinic on the 10th day. Every woman who completed the study experienced relief of symptoms, which was confirmed by clinical examination. Microbiological assays showed the elimination or reduction of the pathogens. Efficacy was seen in women irrespective of the nature of the causative pathogen. Pessary required wetting before insertion, which women did not always remember. It has since been converted into the tablet form with change of vehicle, which does not require wetting. Moreover, the tablet does not soften on storage at prevalent temperatures in summer, making its use feasible in homes which do not have refrigerators. Although the active principles of pessary and tablet are identical, to confirm safety of the tablet, another round of phase I clinical studies were conducted in 20 women at two centres–the Institute for Research in Reproduction, Mumbai and PGI, Chandigarh. Both studies have shown the complete safety of the *praneem* polyherbal tablet. The tablet has also registered a high acceptability from the users. *Praneem* polyherbal tablets or pessaries are both effective in curing RTIs as per the first phase II studies. Efficacy trials are now being extended to five centres located in different parts of the country.

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