Praneem polyherbal cream and pessaries with dual properties of contraception and alleviation of genital infections

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We describe here a polyherbal preparation, which can be used as cream or pessary. Its formulation has potent spermicial action *in vitro* and its contraceptive efficacy is high in rabbits and monkeys. It has in addition, anti-microbial action on *Chlamydia trachomatis*, *Candida albicans* and *Gardenerella vaginalis*, amongst the vaginal pathogens tested *in vitro* in course of Phase I clinical trials conducted in India and abroad with this formulation.

WOMEN groups have expressed their highest preference for the barrier methods (condoms, spermicidal preparations) for contraception. These demand no systemic intake and are used periodically as and when necessary. R&D worldwide is seeking to improve the quality and characteristics of condoms with respect to its resistance to rupture, thinness to provide optimal sensitivity and colour to make it attractive. A female condom has also been devised which is under clinical trials. Amongst the spermicidals, the most widely used preparations employ Nonoxynol-9, a potent detergent, as the principle active compound. Nonoxynol-9 has high spermicidal activity but has also been found to cause inflammatory reaction and irritation upon repeated use. It has a lethal effect on HIV *in vitro*, but clinical studies in Kenya with preparations containing N-9 have given unexpected results on lack of efficacy of the preparation in prevention of heterosexual transmission of the infection[1]. A cream 'consap' containing reetha saponins as spermicial agent has also been made by Setty *et al.*[2] at CDRI, Lucknow. We discuss here the properties of a polyherbal preparation containing three ingredients, namely, purified extract of neem (Praneem), quinine hydrochloride and saponins extracted from the pericarp of *Sapindus mukerrossi*. These ingredients have synergistic action on killing of sperms (Table 1). They also have simultaneous antimicrobial and antifungal action on several pathogens of the genital tract. The cream and other formulations made from the same ingredients are under clinical trials in India and also in four countries abroad. The findings of these trials are briefly reviewed.

Preparation

Praneem polyherbal cream contains purified extract of neem seeds (Praneem), quinine hydrochloride and saponins from reetha, dispensed in a water-washable cream base. The cream is prepared using pharmaceutically

Table 1. Spermicidal activity of components of Praneem polyherbal cream

Component	Initial concentration (mg/ml)	Highest spermicidal dilution	MEC (mg/ml)
Praneem	250	Neat	250
Reetha saponins	10	22 ± 2.9	0 508 ± 0 05
Quinine HCl	30	10 ± 1 2	3 46 ± 0 30
Combination			
Praneem	250		3 47
Reetha saponin	10	72 ± 8	0 138
Quinine HCl	7 5		0 104
Nonoxynol-9	10	64 ± 10 5	0 186 ± 0 03

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Figure 1. Praneem polyhedral cream and applicator

acceptable ingredients and stabilizers. An essential oil is added to mask the unpleasant odour of the neem extract[3]. An applicator is provided to dispense a measured quantity of the cream (Figure 1).

Spermicidal properties

These preparations have been tested by Sander Cramer slide test[4]. Each one of the three ingredients has spermicidal action. In combination, the total killing of sperms within 20 s was achieved at a lower concentration[5]. The ability of the ingredients to modify the properties of cervical mucus was tested by one end test (OET). Praneem at a concentration of 10% has high potency to modify the mucus and inhibit the penetration of sperms. Double end test[6] (DET) has also been performed for each one of the ingredients individually and in combination to arrive at complementary traits of these ingredients for exercising sperm killing and permeation in the cervical mucus.

Contraceptive efficacy

The ingredients used either in liquid form or as cream were highly effective in preventing pregnancy in rabbits[7] (Table 2). Both the liquid and the cream were also effective in post coital tests in bonnet monkeys and baboons. Baboons mated in mid-cycle with males of proven fertility did not conceive after application of the preparation.

Safety

Acute and sub-acute toxicity studies were carried out on the polyherbal cream at the Postgraduate Institute of Medical Education and Research, Chandigarh. The data in 2 species, namely rodents and monkeys, show the safety of the preparation and lack of side-effects. The toxicology data have been approved by the expert committee of the Indian Council of Medical Research. Also, the standard Draize test on normal and abraded skin of rabbits, 21-days cumulative skin sensitivity test in human volunteers and 14-days cumulative vaginal application in rabbits did not reveal any irritation or sensitization with the cream[8].

The antimicrobial action

The ingredients, individually and in combination, have been evaluated for their action on clinical isolates of *Candida albicans*. One of the ingredients and the total formulation inhibited completely the growth of candida on agar. Another ingredient was strongly reactive against 11 isolates of *Staphylococcus aureus*. These isolates were resistant to four or more antibiotics. The growth of *S. aureus* in culture was completely inhibited below the concentrations employed in the cream. One of the components of the formulation has also been tested for the effect on *Gardenerella vaginalis*, where it exhibits potent antiparasitic effect at lower concentrations than those employed in the formulation.

Clinical trials

With the approval of the Drug Controller of India and Ethical Committee of the Institution, Phase I clinical trials were conducted with the polyherbal cream at Safdarjung Hospital, New Delhi, in patients with abnormal discharge due to genital infections[9]. The subjects were asked to apply 5 ml of the cream every night for 7–14 days. After clinical examination, swabs were taken for detection of five commonly encountered pathogens, namely, *Chlamydia trachomatis*, *Neisseria gonorrohoea*, *Trichomonas vaginalis*, *Gardenerella vaginalis* and *Candida albicans*. The patients reported to the clinic on the 8th day for clinical examination as well as for microbiological investigations. The cream was highly effective in patients with *C. trachomatis* infection, its clearance from cervicovaginal region was observed in every case examined in this hospital. Patients with vaginitis due to candida were also benefited clinically in trials in Delhi. However, most of them remained culture-positive. In contrast to these observations, Vivian Brache and Frank Alvarez at Santo Domingo, Dominican Republic, observed both clinical and microbiological improvement in 5 out of 6 patients

Table 2. Contraceptive efficacy of praneem polyherbal cream is rabbits

Time elapsed between application and mating	No of cycles studied			Percentage conception
	Total	Fertile	Infertile	
Experimental group				
10 min	5	0	5	00
30 min	23	0	23	00
60 min	14	1	13	7 0
90 min	7	2	5	28.6
12 h	4	3	1	75
Control group				
10 min	2	2	0	100
30 min	9	8	1	89
60 min	3	2	1	67

having severe candida infection, after 7 days of use of the cream.

Paulo and Ladipo[10] at Bahia, Brazil, have carried out clinical chemistry, haematology and studied other parameters in subjects before and after 7 daily applications of the cream. The treatment did not produce any significant variation in these values. The cream was well tolerated and no immediate or delayed adverse reactions were reported. More recently, Shaaban *et al.* at Assiut (Egypt) have extended the studies on polyherbal cream under the auspices of 'South-to-South Collaboration in Reproductive Health'. Twenty women with vaginitis were screened for viable microbial count. The most remarkable observation of Shaaban was a marked decrease in viable microbial count (from 10^{14} to 10^{5}) with seven applications. Clinical symptoms were also improved.

Comments

Neem seed extracts have a number of medicinally active ingredients. Amongst these are the components with spermicidal action[11, 12]. The water-soluble polysaccharides are potent stimulators of γ-interferon and other cytokines, which in turn have tumouricidal and antiviral activities[13]. The extracts also contain, in the nonpolar fraction, compounds which activate local cell-mediated immunity. A single application in the uterus prevents fertility for several months without impairment of ovulation or change in sex steroid hormone levels[14]. The action is exercised by activation of phagocytic cells and by expression of MHC Class II antigens. The uterus becomes responsive to sperms, releasing locally the cytokines such as IFN and TNF, which in turn kill the sperms or the developing embryo[15, 16]. Quinine hydrochloride has a wide spectrum of action on microbes and viruses in addition to its spermicidal activity. Saponins from sapindus act as a detergent to potentiate the spermicidal action of the other two ingredients. The triple herbal formulation has thus better spermicidal action than any one of these ingredients used alone. It also embodies the desirable trait of reacting against genital pathogens.

Recent studies indicate a strong action of this preparation against HIV also. These studies, however, are *in vitro* and have to await further testing in various systems as well as in *in vivo* models for transmission of AIDS, before conclusions can be drawn. While the polyherbal cream has ingredients with desirable properties, one of its main drawbacks currently is the strong neem odour. Steps are being taken to improve fragrance and other cosmetic qualities of the preparation by using alternate base and delivery vehicles.

1. Ngugi, E., Kreiss, J., Holmes, K, Ndinya, J., Waiyaki, P, Roberts, P. L, Rumijo, I, Sajabi, R., Kimata, J, Fleming, T. R., Anzala, A., Holton, D. and Plummer, F., in *Barrier Contraceptives. Current Status and Future Prospects* (eds. Mauck, C., Cordero, M., Gabelnick, H L., Speiler, J M. and Rivera, R), Wiley-Liss, New York, 1994, pp 261–264
2. Setty, B S, Kamboj, V P, Garg, H. S. and Khanna, N M, *Contraception*, 1976, **14**, 571.
3. Talwar, G P., Garg, S., Singh, R, Sharma, P. L, Dhar, V, Taluja, V., Dhawan, S and Upadhyay, S N, in *Barrier Contraceptives Current Status and Future Prospects* (eds Mauck, C., Cordero, M, Gabelnick, H. L, Speiler, J. M and Rivera, R), Wiley-Liss, New York, 1994, 273–277
4. Sander, F. V and Cramer, S D, *Hum Fertil*, 1941, **6**, 134
5. Garg, S, Doncel, G, Chabra, S, Upadhyay, S. N and Talwar, G. P, *Contraception*, 1994, in press
6. Doncel, G, in *Barrier Contraceptives Current Status and Future Prospects* (eds Mauck, C, Cordero, M, Gabelnick, H L, Speiler, J. M and Rivera, R), Wiley-Liss, New York, 1994, 147–161
7. Garg, S, Taluja, V., Upadhyay, S N. and Talwar, G P, *Contraception*, 1993, **48**, 591–596
8. Talwar, G P, Upadhyay, S. N, Garg, S, Kaushic, C, Kaur, R and Dhawan, S., in *Neem Research and Development* (eds Randhawa, N S and Parmar, B S), Publication No. 3, Society of Pesticide Science, India, 1993, 227 234
9. Garg, S, Kaur, R, Upadhyay, S N and Talwar G P, in *Barrier Contraceptives Current Status and Future Prospects* (eds Mauck, C, Cordero, M, Gabelnick, H L, Speiler, J M and Rivera, R), Wiley-Liss, New York, 1994, 283 287

10. Ladipo, O A, in *Barrier Contraceptives: Current Status and Future Prospects* (eds Mauck, C., Cordero, M, Gabelnick, H L, Speiler, J M and Rivera, R), Wiley-Liss, New York, 1994, pp 277–283
11 Riar, S S, Devakumar, C., Ilavazaghan, G, Bardhan, J, Kain, A K, Thomas, P., Singh, R and Singh, B, *Contraception*, 1990, **42**, 479–487
12 Talwar, G. P., Upadhyay, S N, Kaushic, C., Singh, A. and Sharma, M G, 1993, US Patent No 5196197.
13 Fujiwara, T., Sugishta, E., Takeda, T, Oghihara, Y, Shimiju, M, Nomura, T and Tomita, Y., *Chem. Pharm. Bull.*, 1984, **32**, 1385–1391
14 Upadhyay, S. N, Kaushic, C., Talwar, G. P, *Proc R Soc London B*, 1990, **242**, 175–179
15. Upadhyay, S. N, Dhawan, S., Garg, S. and Talwar, G P, *Int J. Immunopharmacol.*, 1992, **14**(7), 1187–1193.
16. Talwar, G. P, Pal, R, Dhawan, S, Singh, O. and Shaha, C., in *Population The Complex Reality*, A report on the population summit of the world's scientific academies (ed Grahm, S. F.), 1993, The Royal Society, London

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Zona pellucida as a target for immunocontraception

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The zona pellucida (ZP) has generated considerable interest as a target for the immunocontraceptive vaccine, blocking pregnancy at pre-fertilization stage. Antibodies against porcine ZP3 have been shown to inhibit sperm–egg interaction. The immunological cross-reactivity among the various species of ZP glycoproteins, has led to the possibility of heterologous immunization. Recent studies in non-human primates using purified ZP components showed reversible infertility without side effects. More recently, peptide immunogens based on the ZP sequence, have become candidate contraceptive vaccines with the demonstration that the deglycosylated ZP components can block fertility with reduced ovarian dysfunction.

KEEPING in view the present rate of growth, it is projected that the human population will cross 6.3 billion mark by 2000 AD and 10–12 billion by 2050 AD. Moreover, most of this population explosion would be in the developing world, further worsening the living conditions in these countries. This has necessitated the need to develop newer and safer methods of contraception. Vaccines regulating fertility are recent entrants in this field. Immunocontraception entails generating either humoral (antibody) or cell-mediated immune response (CMI) against the molecule(s) having a crucial role in the process of reproduction. An ideal immunocontraceptive vaccine should be (i) effective in preventing conception, (ii) potentially reversible, (iii) available in large quantities at reasonable expense, and (iv) free of any side effects. Such vaccines can act at either pre- or post-fertilization stage. To design immunocontraceptive vaccines aimed at blocking at pre-fertilization stage, antigens pertaining to spermatozoa and egg are being investigated along with other candidates such as gonadotropin releasing hormone (GnRH) and ovine follicle stimulating hormone (oFSH). Amongst the egg antigens, zona pellucida has generated considerable interest[1,2]. The zona pellucida is unique among immunocontraceptive targets by virtue of its location within the female reproductive system, and represents a structure through which sperm must pass to fertilize the ovum. Moreover, the expression of zona pellucida genes is highly specific to the oocyte.

Zona pellucida glycoproteins

The zona pellucida (ZP), an acellular layer surrounding the mammalian oocyte and pre-implantation embryo is formed by the organization of 3–5 families of acidic glycoproteins in most of the species studied[3]. These have been separated by gel electrophoresis or column chromatography. Under non-reducing condition, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of murine ZP reveals three families of glycoproteins ZP1 (200 kDa), ZP2 (120 kDa) and ZP3 (83 kDa)[4]. The human ZP shows ZP1 (90–110 kDa), ZP2 (64–78 kDa) and ZP3 (57–73 kDa)[5]. The porcine ZP shows ZP1 (80–90 kDa) and ZP3 (55 kDa)[6]. The porcine ZP under reducing condition resolves into ZP1 (82 kDa), ZP2 (65 kDa), ZP3 (55 kDa) and ZP4 (21 kDa). Porcine ZP1 revealed immunological cross-