ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 1 Issue 5

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

A Review on Spermicidal Activities of *Azadirachta indica*

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In recent year, there were various types of contraceptives developed having different mode of action. These contraceptives are prepared from natural as well as synthetic sources. These contraceptives worked by prevent the fusion of sperm into ovum, change female hormonal levels and spermicidal activity. Many compounds with different pharmacological activity have been evaluated in vitro for their spermicidal activity. *Azadirachta indica* showed various pharmacological activities and have demonstrated to possess good spermicidal activity also. But there is still a need to develop alternative compounds for future use as safe spermicide

Keyword: Spermicidal, *Azadirachta indica*, Antifertility Agents

1. Introduction

Preparations of plants or parts of them were widely used in popular medicine since ancient times and till today the use of phytomedicines is widespread in most of the world's population[1].

Herbs are used for thousands of centuries by many cultures for their medicinal values. Herbal treatment is very popular because it is easily available, cheap and less toxic. *Azadirachta indica* (neem) is a herbal plant widely distributed in our subcontinent during all seasons. For thousands of years the beneficial properties of *A. indica* A. Juss have been recognized in the Indian traditional medicine. Each part of the neem tree has some medicinal property. Neem leave, bark extracts and neem oil are commonly used for therapeutic purpose (*Tewari, 1992*). Various biological activities showed by neem compounds, neem extracts and reasonable medicinal applications along with their safety evaluation. Neem has two closely related species: *A. indica* A. Juss and *Melia azedarach* Linn. (dharek), the former is popularly known as Indian neem (margosa tree) or Indian lilac, and the other as the Persian lilac. Neem has been extensively used in ayurveda, unani and homoeopathic medicine. The Neem is still regarded as 'village dispensary' in India or a tree for solving global problems'. More than 135 compounds have been isolated from different parts of neem with the chemical and structural diversity. These compounds have been divided into two major classes: isoprenoids (like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C-secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins or amino acids and carbohydrates (polysaccharides), sulphur compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc (*Biswas et al., 2002*). Nimbidin, a major bitter component of

seed kernels oil of *A. indica* demonstrated several biological activities. From crude components some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated.

1.1 Medicinal Uses

Almost all parts of the neem tree have been used as traditional Ayurvedic, unani and sidhha medicine in India. Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation, blood purifier and also as a general health tonic. It also used for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer. Neem oil used to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis. Neem contained various compounds which showed various biological activities such as anti-inflammatory; Antiarthritic; Antipyretic; Hypoglycaemic; Antigastric ulcer; Spermicidal; Antifungal; Antibacterial; Diuretic; Antimalarial; Antitumour; Immunomodulatory etc. (Table 1).

Table 1: Pharmacological activity of various parts of Neem

S. No.	Part used	Medicinal properties
1	Leaves	Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcer
2	Barks	Analgesic, alternative and curative fever
3	Flowers	Bile suppression, elimination of intestinal worms and phlegm
4	Fruits	Piles, intestinal worms, urinary disorder, epistaxis, phlegm, eye problem, diabetes, wounds and leprosy
5	Twings	Cough, asthma, piles, phantom tumor, intestinal worms, spermatorrhoea, obstinate urinary disorder, diabetes
6	Gum	Scabies, wounds, ulcers, skin diseases
7	Seeds	Leprosy and intestinal worms
8	Oil	Leprosy and intestinal worms

The aqueous extract of neem bark and leaf also possesses anticomplement and immunostimulant activity. Neem oil has been shown to possess activity by selectively activating the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge. Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits. Neem leaf and bark aqueous extracts produce highly potent anti-acid secretory and antiulcer activity. Intra-vaginal application of neem oil, prior to coitus, can prevent pregnancy. It could be a novel method of contraception. Oil from the leaves, seed and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including M. tuberculosis and streptomycin resistant strains. *In-vitro*, it inhibits *Vibrio cholerae Klebsiella pneumoniae, M. tuberculosis* and *M. pyogenes*. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *S. faecalis*. Neem seed and leaf extracts are effective against both choroquin-resistant and sensitive strain malarial parasites. Extracts of neem leaf, neem oil seed kernels are effective against certain fungi including Trichophyton, Epidermophyton, Microspor Trichosporon, Geotricum and Candida. Aqueous leaf extract offers antiviral activity against *Vaccinia* virus, *Chikungemya* and *measles* virus. Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7, 12-dimethylbenz[a] anthracene (DMBA), as revealed by reduced incidence of neoplasm. Neem may exert its chemopreventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes. The antioxidant activity of neem seed extract has been demonstrated in vivo during horse- grain germination. Varying degrees of central nervous system (CNS) depressant activity

in mice was observed with the leaf extract. Fractions of acetone extract of leaf showed significant CNS depressant activity. Neem oil suppresses various species of pathogenic bacteria such as *S. aureus, S. typhosa, S. paratyphi, V. cholerae* and all strains of *M. tuberculosis* (*Chaurasia and Jain, 1978; Rao et al., 1986; Rao, 2005*). Efficacy of NIM-76, a spermicidal fraction from neem oil showed potent broad spectrum antimicrobial activity against certain bacteria, fungi and poliovirus (*SaiRam, 2000*). Neem may be used for its easy availability and significant effect against bacteria. Neem oil was prepared by steam distillation process and its effect against *S. aureus, S. typhi*, *E. coli* and *P. aeruginosa*. The MIC against *S. aureus*, *S. typhi*, *E. coli* and *P. aeruginosa* was at 1:32, 1:16, 1:32 and 1:8 dilution. The average diameter of zone of inhibition against *S. aureus* with neem oil was 19 mm whereas it was 30 mm with cefepime. *S. typhi, E. coli* and *P. aeruginosa* exhibited zone of inhibition. Among all test bacteria *S. aureus* had lowest MIC. *In-vitro* antibacterial activity of neem oil showed 92% susceptibility against *P. aeruginosa, S. pyogenes, E. coli, Proteus* group and *K. aerugenes*. The MICs varying between ¼ to 1/64 dilution (*Jahan et al., 2007*). Neem extract is effective to cure ringworm, eczema and scabies. Lotion derived from neem leaf, when locally applied, can cure these dermatological diseases within 3-4 days in acute stage or a fortnight in chronic case. A paste prepared with neem and turmeric was found to be effective in the treatment of scabies. Neem leaf extract has been prescribed for oral use for the treatment of malaria by Indian ayurvedic practitioners from time immemorial. Recently, a clinical trial has been showed the efficacy of neem extract to control hyperlipidemia in a group of malarial patients severely infected with *P. falciparum*. The lipid level, especially cholesterol, was found to be lower during therapy when compared to non-malaria patients. Reports are available regarding the use of neem to treat patients suffering from various forms of cancer. One patient with parotid tumour and another with epidermoid carcinoma have responded successfully when treated with neem seed oi1. NIM-76, a refined product from neem oil, where intra-vaginal application before sexual intercourse could prevent pregnancy with no adverse effect on vagina, cervix and uterus. The data suggested that intrauterine treatment is safe. Various studies have been reported on the safety evaluation of different parts of neem as well as its various biologically active products. Nimbidin produces sub-acute toxicity in adult rats after daily administration of 25, 50 or 100 mg/kg for six weeks. A significant hypoglycaemic effect was observed by feeding nimbidin to fasting rabbits. Nimbidin also has spermicidal activity. Nimbolide, a major chemical component of neem seed oil, and nimbic acid were found to be toxic to mice when given intravenously or intraperitoneally. They are, however, less toxic to rats and hamster. Nimbolide and nimbic acid at a lethal dose cause death in most animals by dysfunction of kidney, small intestine and liver as well as by marked and sudden drop of arterial blood pressure.

1.2 Sexually transmitted diseases and Overview

While relatively few researchers have focused on neem efficacy in treating sexually transmitted diseases, the reports that have been completed are overwhelmingly positive. The most exciting study, focused on both the treatment and prevention of AIDS. Ten volunteers received 1000 grams of neem bark extract daily for 30 days, resulting in significantly increased CD4+ cell counts, body weight and hemoglobin counts. In fact, researchers report that all AIDS-related conditions were completely resolved during the treatment period and month-long follow-up with no adverse effects. Concurrent in vitro studies showed that neem bark extract prevented cytoadhesion of the HIV virus in 75% of protected cells, compared to 100% infection in unprotected cells. Similar results were reported with malaria and cancer cells. Previous studies at Johns Hopkins University had shown that neem demonstrated activity against the virus that causes genital herpes both in vitro and in mice. Ongoing reports from India indicate that neem oil in a spermicide has protective agents against the fungus that causes yeast infections and thrush and

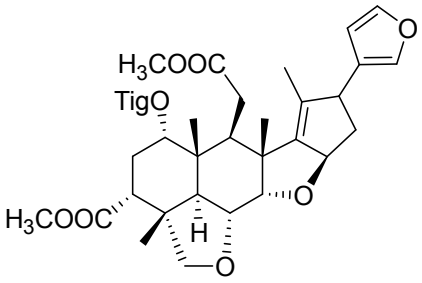
a private company has begun clinical tests on a neem-based spermicide that it hopes will also control the spread of AIDS. And while multiple reports detailing neem's immunostimulatory and contraceptive properties don't necessarily address sexually transmitted diseases, they may play a role in research over the next few years.

1.3 History of Spermicides

The first written record of spermicide use is found in the Kahun Papyrus, an Egyptian document dating to 1850 BCE. It described a pessary of crocodile dung and fermented dough. It is believed that the low pH of the dung may have had a spermicidal effect. Further formulations are found in the Ebers Papyrus from approximately 1500 BCE. It recommended mixing seed wool, acacia, dates and honey, and placing the mixture in the vagina. It probably had some effectiveness, in part as a physical barrier due to the thick, sticky consistency, and also because of the lactic acid (a known spermicide) formed from the acacia. Writings by Soranus, a 2nd century Greek physician, contained formulations for a number of acidic concoctions claimed to be spermicidal. His instructions were to soak wool in one of the mixtures, then place near the cervix. Spermicide is a contraceptive substance that eradicates sperm, inserted vaginally prior to intercourse to prevent pregnancy. As a contraceptive, spermicide may be used alone. However, the pregnancy rate experienced by couples using only spermicide is higher than that of couples using other methods. Usually, spermicides are combined with contraceptive barrier methods such as diaphragms, condoms, cervical caps, and sponges. Combined methods are believed to result in lower pregnancy rates than either method alone (*Connell. 1991*). Spermicides are unscented, clear, unflavored, non-staining, and lubricative. Spermicides cause irritation and according to the CDC, studies have shown that spermicides increase the risk of HIV. Contraceptive Technology states that spermicides have a failure rate of 18% per year when used correctly and consistently, and 29% under typical use. Extractives of the neem plant such as neem oil have also been proposed as spermicides based on laboratory studies. Animal studies of creams and pessaries derived from neem have shown they have contraceptive effects, however trials in humans to determine its effectiveness in preventing pregnancy have not yet been conducted.

1.4 Azadirachta indica:

The neem tree, *A. indica* is indigenous to the Indian subcontinent. Neem oil, extracted from the seeds of the neem tree, has been found to possess strong spermicidal activity. By the process of hydrodistillation, the volatile fraction of neem oil has been isolated and coded as NIM-76. A concentration of 25 mg/mL of the compound was found to achieve total spermicidal effect in 20 sec. Vaginal irritation study conducted in rabbits, by intravaginal application of 15 mg of NIM-76 in 2 mL of gelatin jelly for 10 days showed no irritation to the vaginal mucosa. The aqueous extract of old and tender neem leaves is a potent spermicide. The minimum effective concentration required to kill 1 million sperm in 20 sec was 2.91 mg and 2.75 mg for tender and old leaf extract, respectively (Khillare. and Shrivastav. 2003; Shah et al., *2008*). Salannin (**12**), a limonoid bitter principle of the seed oil of *A. indica*, showed antiulcer, antibacterial and spermicidal activities [Ankita et al., 2010].



Praneem polyherbal formulations containing purified extracts of *A. indica* have shown activity against HIV and sexually transmitted disease pathogens in studies in vitro. The product also has contraceptive properties. This has prompted its development as a possible microbicide. We evaluated the safety of Praneem polyherbal tablet use among HIV-uninfected women. Twenty

eligible women were enrolled in a Phase I open-label study requiring 14 days of consecutive intravaginal use of Praneem polyherbal tablets. Nine (45%) participants experienced 17 episodes of genital irritation. Transient genital itching was reported by eight (40%) participants, burning micturation by two (10%) and lower abdominal pain, genital burning and intermenstrual spotting by one (5%) each. On colposcopy, petechial haemorrhage was observed in two participants, one on day 7 and the other on day 14, and both were resolved without any treatment. There were no serious adverse events. Praneem polyherbal tablets were found to be safe for once daily intravaginal use for 14 consecutive days in sexually active HIVuninfected women and a Phase II study may be taken up as a priority (*Joshi et al., 2005*).

The seed oil of *A. indica* A. Juss (neem) is used in traditional medicine for its antidiabetic, spermicidal, antifertility, antibacterial, and wound healing properties. The present study was undertaken to investigate the quantitative aspects of follicular development in cyclic female albino rats after oral administration of polar and non-polar fractions of *A. indica* seed extract at 3 and 6 mg kg body weight-1 day-1 for 18 days. The extracts were dissolved in olive oil to prepare doses on a per kg body weight basis. There was a significant reduction (P=0.05) in the number of normal single layered follicles (*A. indica:* 0.67+/0.33 and 4.67+/-2.03 after 3 and 6 mg/kg nonpolar fraction and 3.33+/-1.67 and 1.00+/-1.00 after 3 and 6 mg/kg polar fraction vs control: 72.67+/-9.14 after 24 mg/kg polar and non-polar fractions, vs control: 73.40+/-7.02) and follicles in various stages (I-VII) of follicular development in all treatment groups. These extracts also significantly reduced (P= 0.05) the total number of normal follicles in the neem (14.67+/-5.93 and 1.00+/- 1.00 after 3 and 6 mg/kg polar and 3.67 +/- 0.88 and 5.33 +/- 2.03 after 3 and 6 mg/kg non polar fraction) treatments compared to control (216.00 +/-15.72 and 222.20+/-19.52). Currently, indiscriminate use of persistent and toxic rodenticides to control rodent populations has created serious problems such as resistance and environmental contamination. Therefore, it becomes necessary to use ecologically safe and biologically active botanical substances that are metabolized and are not passed on to the next trophic level, and that interfere with the reproductive potential particularly growth and differentiation of follicles. This may help elevate the socio-economic status of the country. Thus, the present study is an attempt to investigate the effects of A. indica seed extracts on reproduction of albino rats (*Roop et al., 2005*).

An acetone-water neem leaf extract with antimalarial activity was evaluated in vitro at 5 microg/ml for inhibition of adhesion of malaria parasite-infected erythrocytes and cancer cells to endothelial cells, and at 10 microg/ml for protection of lymphocytes against invasion by HIV. The extract was also evaluated in 10 patients with HIV/AIDS at 1000 mg daily for 30 d. The mean binding of infected erythrocytes and cancer cells per endothelial cell was 15 and 11 respectively in the absence of the extract, and 0 and 2 respectively in with the extract. In the absence and presence of the extract, 0% and 75%, respectively, of lymphocytes were protected. In the treated patients, haemoglobin concentration, mean CD4+ cell count and erythrocyte sedimentation rate, which were initially 9.8 g/dl, 126 cells/microl and 90 mm/h respectively, improved to 12.1 g/dl, 241 cells/microl and 49 mm/h. Mean bodyweight and platelet count, initially 57 kg and 328 x 10(3)/mm3 respectively, increased to 60 kg and 359 x 10(3)/mm3. No adverse effects were observed during the study. The extract showed antiretroviral activity with a mechanism of action that may involve inhibition of cytoadhesion. The results may help in the development of novel antiretroviral and antimalarial drugs (Udeinya et al., 2004).

The main component of this pill is 2-Octadecanoic acid-4-Palmitic acid-2, 4-Pentanediyl ester separated from chloroform extract of neem oil. The microcapsules coated by the re-curdle method were fabricated with an average particle size of 100-180 microm. The morphological characteristics, incorporation

efficiency, carrier reclamation efficiency of the microcapsule were investigated. Kunming mice were used in the experiment, and the anti-fertility effect of the microcapsule on the histology and apoptosis was studied by light and electron microscopy and the flow cytometry. The data obtained clearly indicated that the microcapsule could lead to the payload of medicine, the incorporation efficiency being 90%. After the microcapsules were given to the male mice orally, its anti-fertility effect came into being and could keep the mice in a state of reversible infertility for a long time. The results of histological study and flow cytometry indicate that the mechanism of its anti-fertility effect involves mainly the inhibition of sperm motility and the arrest of spermatogenic process (*Yin et al., 2004*).

The effective concentration of aqueous extract of old and tender *A. indica* (neem) leaves to immobilize and kill 100% human spermatozoa within 20s by Sander-Cramer test. Under the test conditions, minimum effective spermicidal concentrations for tender and old leaf extracts were 2.91 +/- 0.669 mg/million sperm and 2.75 +/- 0.754 mg/million sperm, respectively. No change was observed in morphology of head, mid-piece and tail and no viable sperm seen. The leaf extracts were found to be water soluble and carbohydrate in nature. The effect of different concentrations of extracts (old and tender) on percentage motility of the sperm was also studied. With an increase in concentration, there is a linear decrease in percentage motility, becoming zero at a 3-mg dose within 20s (*Khillare and Shrivastav. 2003*).

Efficacy of NIM-76, a spermicidal fraction from neem oil, was investigated for its antimicrobial action against certain bacteria, fungi and Polio virus as compared to whole neem oil. The NIM-76 preparation showed stronger anti-microbial activity than the whole neem oil. It inhibited growth of various pathogens tested including Escherichia coli and Kleibsiella pneumoniae which were not affected by the whole neem oil. NIM-76 also exhibited antifungal activity against Candida albicans and antiviral activity against Polio virus replication in vero cell lines. It also protected mice from systemic candidiasis as revealed by enhanced % survival and reduced colony forming units of C. albicans in various tissues. This shows that NIM-76 has a potent broad spectrum anti-microbial activity (SaiRam et al., 2000).

Neem seed and leaf extracts have immunomodulators that induce cellular immune reactions. The oral administration of the neem seed extracts in rodents and primates could completely abrogate pregnancy at an early post implantation stage. Complete restoration of fertility was observed in the animals treated in the subsequent cycles. For the purpose of using neem as a long term contraceptive, an activity guided fractionation. Sequentially extracted fractions of neem seeds were tested orally at an early post implantation stage in rats. The hexane extract of the neem seeds was found to be biologically active. The active fraction, identified as a mixture of six components, could completely abrogate pregnancy in rodents up to a concentration of 10%. No apparent toxic effects could be seen following treatment with the fraction. The treatment with the active fraction caused a specific activation of T lymphocyte cells of CD8+ subtype as well as phagocytic cells followed by elevation in cytokines gamma-interferon and TNF. A pure active fraction of neem seeds could be obtained for the purpose of early post implantation contraception when given orally, and its mechanism of action seems to be by activating cell mediated immune reactions (*Mukherjee et al., 1999*).

Subsequently, hexane extract was sequentially fractionated through the last active fraction using various separation techniques and tested for antifertility activity at each step. The active fraction was identified by HPLC to be a mixture of six components, which comprises of saturated, mono and di-unsaturated free fatty acids and their methyl esters. Dose response study was performed with the last active fractions. The antifertility activity with the active fraction was

reversible in nature and it was completely active until 5% concentration. There was no systemic toxic effect following the administration of the active fraction. This study, proposes an active fraction from neem seeds, responsible for long term and reversible blocking of fertility after a single intrauterine administration with high efficacy (*Garg et al., 1998*).

Microbicide candidates were selected that have demonstrated activity against sperm or sexually transmitted disease pathogens in vitro, and the efficacy of these agents for preventing vaginal transmission of genital herpes infection was evaluated in the progestin-treated mouse. Each agent was delivered to the vaginas of mice approximately 20 sec prior to delivering a highly infectious herpes simplex virus-2 inoculum. The following agents provided significant protection: anti-HSV monoclonal antibodies III-174 and HSV8, modified bovine betalactoglobulin (beta-69), carrageenan, concanavalin A, chlorhexidine, dextran sulfate (average molecular weight 8,000 and 500,000), fucoidan, neem, nonoxynol-9, polystyrene sulfonate, and povidone-iodine. Two agents, gramicidin and heparan sulfate, though highly effective in vitro, were not protective in vivo at the doses tested (*Zeitlin et al., 1997*). *A. indica* seed and leaf extracts have spermicidal, anti-microbial, anti-fungal and anti-viral properties. They are also immunomodulators that induce primarily a TH1 type response. These properties are being exploited to develop two different useful methods of fertility control. Neem extracts given orally at early post-implantation stage terminate pregnancy in rodents and primates. Treatment has no residual permanent effect and fertility is regained in subsequent cycles. The mechanism by which the action occurs is not fully clear. A transient increase in CD4 and more significantly in CD8 cells is noticed in mesenteric lymph nodes and spleen. A rise in immunoreactive and bioactive TNF-α and IFN-γ in draining lymph nodes, serum and foetal-placental tissue is observed. A polyherbal cream and pessary have been developed containing three active ingredients of plant origin. These have synergistic spermicidal properties on human sperm as determined by the Sander Cramer test. Their use before mating has high contraceptive efficacy in rabbits and baboons. Another interesting property is their inhibitory action on a wide spectrum of micro-organisms, including *Candida albicans*, *C. tropicalis, Neisseria gonorrhoeae,* the multidrug-resistant *Staphylococcus aureus* and urinary tract *Escherichia coli, Herpes simplex-2* and *HIV-1*. Phase I clinical trials have been completed in India, Egypt and the Dominican Republic, and indicate the safety of the formulation, its acceptability and beneficial action invaginosis due to infections (*Talwar et al., 1997*). Microbicide candidates were selected that have demonstrated activity against sperm or sexually transmitted disease pathogens in vitro, and the efficacy of these agents for preventing vaginal transmission of genital herpes infection was evaluated in the progestin-treated mouse. Each agent was delivered to the vaginas of mice approximately 20 sec prior to delivering a highly infectious herpes simplex virus-2 inoculum. The following agents provided significant protection: anti-HSV monoclonal antibodies III-174 and HSV8, modified bovine betalactoglobulin (beta-69), carrageenan, concanavalin A, chlorhexidine, dextran sulfate (average molecular weight 8,000 and 500,000), fucoidan, neem, nonoxynol-9, polystyrene sulfonate, and povidone-iodine. Two agents, gramicidin and heparan sulfate, though highly effective in vitro, were not protective in vivo at the doses tested (Zeitlin et al., 1997). To develop a self-administered, orally delivered method for abrogation of early pregnancy. Use of purified Neem extracts containing immunomodulators stimulating Th1 cells and macrophages; test animals, rats, baboons, and monkeys, onset of pregnancy confirmed by surgery and counting of implants on day 7 in rats and by chorionic gonadotropin (CG) and progesterone assays in primates; termination defined by complete resorption on day 15 in rats and by bleeding and decline of CG and progesterone in baboons. Pregnancy was terminated successfully in both rodents and primates with no significant side effects. Fertility was regained in both species after one or two

irregular cycles. Progeny born had normal developmental landmarks and mothered normal litters in the course of time. The active principle in Neem has been partially fractionated by activity-guided purification. A cascade of events are involved in abrogation of pregnancy. In primates, a decrease in progesterone is an early event. A transient increase in CD4 and CD8 cells is noted in spleen at 96 hrs. and in mostly CD8 cells in mesenteric lymph nodes. Treatment causes an elevation of both immunoreactive and bioactive TNF-alpha and gamma-interferon in serum, mesenteric lymph nodes, and foetoplacental tissue. Immunomodulators of plant origin are potentially usable for termination of unwanted pregnancy (*Talwar et al., 1997*). Neem (*A. indica*) seed and leaf extracts have spermicidal, anti-microbial, anti-fungal and anti-viral properties. They are also immunomodulators. These properties are being exploited to develop two different useful methods of fertility control. Neem extracts given orally at early post-implantation stage terminate pregnancy in rodents and primates. Treatment has no residual permanent effect and fertility is regained in subsequent cycles. A transient increase in CD4 and more significantly in CD8 cells is noticed in mesenteric lymph nodes and spleen. A rise in immunoreactive and bioactive TNF-α and IFN-γ in draining lymph nodes, serum and foetal-placental tissue is observed. A polyherbal cream and pessary have been developed containing three active ingredients of plant origin. These have synergistic spermicidal properties on human sperm. Their use before mating has high contraceptive efficacy in rabbits and baboons. Another interesting property is their inhibitory action on a wide spectrum of micro-organisms, including *Candida albicans, C. tropicalis, Neisseria gonorrhoeae,* the multidrug-resistant *Staphylococcus aureus* and urinary tract *Escherichia coli,* Herpes simplex-2 and HIV-1. Its beneficial action in invaginosis that is due to infections (Talwar et al., 1997). The immunomodulatory properties of NIM-76 have been described in this paper. Pre-treatment of rats with a single i.p. injection of NIM-76 resulted in an increase in polymorphonuclear (PMN) leukocytes with a concomitant decrease in lymphocyte counts. The immunomodulatory activity of NIM-76 was found to be concentration-dependent. At 120 mg/kg body weight, there was an enhanced macrophage activity and lymphocyte proliferation response, while the humoral component of immunity was unaffected. At higher concentrations of NIM-76 (300 mg/kg body weight), there was a stimulation of mitogen-induced lymphocyte proliferation, while macrophage activity remained unaffected. However, a fall in primary and secondary antibody titres was observed. The study indicates that NIM-76 acts through cell-mediated mechanisms by activating macrophages and lymphocytes (*SaiRam et al., 1997*).

The present study was undertaken to elucidate the mechanism of spermicidal action of NIM-76, a fraction isolated from neem oil. The spermicidal activity of NIM-76 was confirmed using a fluorescent staining technique. NIM-76 was found to affect the motility of the sperm in a dose-dependent manner. Supplementation of pentoxifylline, which is known to enhance the motility of the sperm, could not prevent the spermicidal action of NIM-76. There was a gradual leakage of cytosolic LDH from the sperm in the presence of NIM-76. Electron microscopic studies revealed the formation of pores and vesicles over the sperm head, indicating the damage to the cell membrane. Membrane fluidization studies did not reveal any significant change in the fluidity of sperm cell membrane structure. PIP: Neem oil, an extract of a native plant of India, has been demonstrated to have antifertility, anti-implantation, and abortifacient properties. An active fraction, termed NIM-76, was extracted that eliminates its abortifacient properties while retaining spermicidal activity. This fraction kills all human sperm in vitro in under 20 seconds at a concentration of 25 mg/ml. With increases in NIM-76 concentrations from 10 to 1000 mcg/ml, there was a linear decrease in percentages of motile as well as progressively motile sperm with time; also recorded were decreases in percentages of rapid, medium, and slow moving sperm, mean track speed,

progressive velocity, mean linearity, and lateral head displacement and an increase in the percentage of static sperm. Electron microscopy revealed the formation of pores and vesicles over the sperm head, indicating damage to the cell membrane. Membrane fluidization studies did not reveal any significant change in the fluidity of sperm cell membrane structure. Since calcium supplementation did not relieve the sperm from the spermicidal action, it was determined that NIM-76 does not cause any depletion of intracellular calcium. The capability of NIM-76 to selectively kill sperm without affecting normal cells makes it a highly desirable potential vaginal contraceptive agent (Sharma et al., 1996). The aim of this study was to find out the role and mechanism of action of neem oil as a postcoital fertility blocker in mouse. Female mice were injected with neem oil (20 or 40 microliters) surgically into each uterine horn on day 2 postcoitum (pc). Both the uterine horns of each mouse were injected. Arachis oil served as vehicle control. Pregnancy success was determined by the number of implanted embryos on day 8 pc and the number of live fetuses in the uteri on day 18 pc. Transforming growth factor-alpha (TGF alpha), epidermal growth factor (EGF), and epidermal growth factor receptor (EGFR) were immunolocalized in the paraffin-embedded sections of the uteri at 0600 hr on day 5 pc. The unimplanted embryos were assessed in the uteri at 2000 hr on day 5 pc. Uterine secretions were assessed for the leukocytes infiltration on day 4 through day 8 pc. The number of implantation sites on day 8 pc and the number of live fetuses on day 18 pc were lower in the neem oil-treated animals compared to their respective control animals at both the concentrations of neem oil (20 and 40 microliters/uterine horn). Neem oil also caused resorption of some embryos between day 8 pc and day 18 pc. In neem oil-treated mice, EGFR immunostaining decreased in the luminal and glandular epithelium and increased in the stroma as determined at 0600 hr on day 5 pc. Uterine secretions on day 4 through day 6 pc from the neem oil-treated mice showed massive leukocyte infiltration. Unimplanted preimplantation embryos, underdeveloped, degenerated, or at blastocyst stage, were recovered from the uteri after flushing at 2000 hr on day 5 pc from the neem oil-treated animals. A number of retrieved unimplanted embryos showed the direct attachment of the leukocytes to their zona pellucida. It is believed that the secretions of these leukocytes might be responsible for the underdevelopment of the early embryos and hence inhibition of implantation. The exact interaction of these leukocytes and their secretions with the early embryos is under investigation. Postcoital intrauterine treatment of neem oil during preimplantation period causes fertility block in mouse by lowering the EGFR localization in the luminal and glandular epithelium, by causing massive leukocytes infiltration into the uteri, by degenerating the early embryos, and by causing the postimplantation embryonic resorptions in the uteri. The possible mechanism of action of neem oil is discussed (Juneja et al., 1996). The use of neem (*A. indica*) seed extracts (Praneem) given orally for abrogation of pregnancy in subhuman primates is described. Oral administration of Praneem was initiated after confirmation of pregnancy using Leydig cell bioassay estimating rising levels of chorionic gonadotropin (CG) in the blood from day 25 onwards of the cycle and continued for six days. Termination of pregnancy was observed with the appearance of blood in the vaginal smears and decline in CG and progesterone. Pregnancy continued in the control animals treated with peanut oil at the same dose. The effect was observed in both baboons and bonnet monkeys. The treatment was well tolerated; blood chemistry and liver function tests had normal values. The animals regained their normal cyclicity in the cycles subsequent to Praneem treatment (Mukherjee et al., 1996).

Praneem Vilci (PV), purified neem oil was reported to exercise a reversible antifertility effect after a single intrauterine instillation in rodents and primates without any adverse effects. After toxicology, drug regulatory and ethical clearances, a phase I clinical trial was conducted on PV. Eighteen healthy tubectomised women were enrolled to evaluate the safety of a single

intrauterine instillation of PV and to determine the effect of its co-administration on anti-hCG response to the heterospecies dimer (HSD) hCG vaccine. Eight women received PV alone and ten women were given the HSD-hCG vaccine in addition. Base-line and post-treatment haematological and biochemical profiles were determined as also the mid-luteal serum progesterone. Endometrial biopsies were examined to assess ovulatory status and the effect of intrauterine treatment with PV on the endometrium. Anti-hCG antibody titres were estimated in women who were concurrently immunized with the HSD vaccine. No untoward reaction was observed in any woman. Menstrual pattern and ovulatory status remained unaltered. Endometrial biopsy after PV instillation in one woman showed non-specific endometritis but she remained asymptomatic. Mild eosinophilia was seen in two women and this reverted to normal on its own. All women receiving PV and the HSD vaccine generated antibodies against hCG. Our data show that intrauterine administration of PV is safe and does not prevent the antibody response to HSD-hCG vaccine (*Talwar et al., 1995*). The mode of antifertility action of intrauterine neem treatment (IUNT) was studied. The effect of IUNT on ovarian functions and uterine responsiveness to ovarian hormones was examined in adult Wistar rats. The treated animals had normal reproductive cycles as indicated by the vaginal smears; serum progesterone levels were also in the normal range. Effect of exogenous estradiol following IUNT in ovariectomized rats showed comparable uterine weight gain as in control group; decidual cell reaction of the uterine epithelium following IUNT was also similar to that of control, indicating normal uterine responsiveness to ovarian hormones. Unilateral IUNT followed by mating resulted in degeneration of embryos on the treated side as noted between days 3-5 post coitum; normal embryos were seen on the contralateral side given peanut oil. The study shows that the mode of antifertility action of IUNT is not because of uterine unresponsiveness to the ovarian hormones but is due to impairment of embryo development. The results of this study thus confirm our earlier observations and show further that the antifertility effect of IUNT is at the pre-implantation stage, localized and without any adverse or toxic effect on the fetal development in the contralateral uterine horn of the unilaterally treated rats. The exact mechanism(s) of antifertility action of IUNT is being investigated (Kaushic and Upadhyay. 1995).

A novel approach for immunocontraception by intervention of local cell mediated immunity in the reproductive system by using single intrauterine application (novel method of contraception) of neem oil has been described earlier. The reversible block in fertility was reported to last for 107-180 days in female Wistar rats (*Upadhyay et al., 1990; Upadhyay et al., 1994*). Long term contraceptive effects of intrauterine neem treatment (IUNT) in bonnet monkeys: An alternative to intrauterine contraceptive devices. The identification and characterization of the biologically active fraction from neem seeds (*A. indica* A. Juss. Family Meliaceae) which is responsible for the above activity in adult female Wistar rats. The mechanically extracted oil and solvent extracts of neem seeds have revealed that the antifertility activity was present in constituents of low to intermediate polarity. A hexane extract of neem seeds was reported to be biologically active (Garg et al., 1994). *A. indica* (Neem) seed extracts are known to activate the local cell-mediated immune reactions after a single intrauterine administration, leading to a long term reversible block of fertility. In order to identify and characterize the active fraction responsible for this activity, neem seeds were extracted by both mechanical expression and solvent extraction using a range of polar to non-polar solvents which yielded 3 broad fractions. The mechanically expressed oil was fractionated using different approaches and studied for antifertility activity. The hexane extract and a corresponding column fraction showed potent and reproducible antifertility activity. Other fractions were less stable with regard to reproducibility of effects and composition. It is

our conclusion that for subsequent fractionation to reach the last active fraction, the hexane extract is the most useful starting material (Garg et al., 1994). The in vitro effect of neem oil was studied on the development of mouse two-cell embryos and trophectodermal cell attachment and proliferation. Exposure of two-cell embryos to neem oil concentrations of 0.050-0.500% for 1 hr, 0.010-0.250% for 12 hr, and 0.005-0.100% for 24 hr caused significant inhibition of the formation of total and hatching blastocysts, in a dose-dependent manner. Neem oil at 0.050-0.100% concentrations inhibited, in a dose-dependent manner, the in vitro attachment and proliferation of trophectodermal cells of partially hatching blastocysts cocultured with human endometrial stromal cells monolayers. Neem oil inhibits the development of two-cell embryos and attachment and proliferation of the trophectodermal cells of partially hatching blastocysts in vitro. The study encourages the use of this herbal product as a postcoital contraceptive that warrants further research (Juneja et al., 1994). In order to identify potent spermicidal agents which are free from the side effects of currently available agents, spermicidal activity of purified neem seeds extract (Praneem), reetha saponins and quinine hydrochloride was studied individually and in combination. Sander-Cramer test was used to assess the activity on human sperm. Under the test conditions, minimum effective spermicidal concentrations for Praneem, reetha saponins and quinine hydrochloride were 25%, 0.05% and 0.346%, respectively. At these concentrations, 100% of the sperm were immobilised within 20 seconds. A positive synergistic effect in the spermicidal activity of these components, if used in combination, was observed which implies the use of reduced concentrations of each to bring about the desired action. The selected combination formulated into a suitable dosage form is likely to offer dual benefit of a potent contraceptive and an antimicrobial preparation. Contraceptive researchers in India and the United States used a modified version of the Sander-Cramer test to measure the minimum concentration of purified neem seeds extract, reetha saponins (pericarp of Sapindus fruits), and quinine hydrochloride to kill all sperm within 20 seconds. They wanted to determine the individual and combined action of these potential spermicidal agents on sperm motility and survival. The concentrations needed to effect the death of 100% of human sperm within 20 seconds were 25% for neem oil, 0.05% for reetha saponins, and 0.346% for quinine hydrochloride. A mixture of 25% neem extract, 1% reetha saponins, and 0.75% quinine hydrochloride was spermicidal up to a dilution of 72 times. This dilution was much higher ($p = .0004$) than the highest spermicidal dilution attained by reetha saponins, the most potent component of the mixture. The positive synergistic effect in the spermicidal activity of these components indicates reduced concentrations of each to achieve effective spermicidal activity (0.39% neem oil, 0.015% reetha saponins, and 0.0012% quinine hydrochloride). Reetha saponins contains considerable oleanolic acid or hederagenin, which have a mild detergent effect, inactivating sperm. Quinine chloride strengthens spermicidal activity and antimicrobial activity. Neem extract induces local cell-mediated immunity. Contraceptive developers can formulate the combination of these 3 components either as a cream or pessary (Garg et al., 1994). Immuno-contraception instructs the body to recognize a self-molecule as foreign, so that the body attacks the molecule, thereby effecting contraception. When researchers were developing a contraceptive vaccine, they considered 3 things: the targeted molecule should be crucial for reproduction, it should be transient in nature, and the antibodies against this molecule should not cross-react with other molecules in the body. They have developed a vaccine using beta-human chorionic gonadotropin (hCG). The pregnant woman's body produces beta-hCG. It sustains the corpus luteum for production of progesterone which induces changes in the uterus conducive to implantation of the zygote. Researchers have linked betahCG with a carrier molecule (tetanus toxoid or diphtheria) to induce an immune attack. They have successfully completed phase 2 efficacy trials. This vaccine also protects against tetanus or diphtheria. Animal studies show that it does

not have any harmful side effects and is reversible. The phase 2 trials included women with 2 children cohabiting with fertile partners. Once the antibody titres surpassed the protective threshold, they discontinued contraceptive use and any avoided pregnancies would be attributed to the vaccine alone. The trials exceeded the norm of 750 protected menstrual cycles for the vaccine to be considered efficacious in 1993. Researchers continued to monitor the women until their anti-beta-hCG titres reached a near-zero level. They are now ready to begin phase 3 trials. Logistical obstacles to overcome are a 2 month-lapse between 1st dose and sufficient titres to protect against pregnancy and multiple injections. Neem oil use may provide protection during the lag phase since it stimulates immune cells in the reproductive tract and has embryocidal and spermicidal effects. A single injection of biodegradable microcarrier systems releasing the vaccine may address the problem with multiple doses (*Koshy. 1994*). Antifertility effects of intrauterine neem treatment (IUNT) was studied in bonnet monkeys. A single administration of 1 ml of neem oil by an intrauterine insemination catheter blocked fertility for 7 to 12 months. The effect was, however, reversible as all the animals became pregnant subsequently and delivered normal babies. The neem oil treatment had no adverse effect on menstrual cyclicity and ovarian functions. The uterus of neem-treated animals showed normal morphology. Immunohistological studies, however, demonstrated a significant increase in the number of MHC-II antigen-positive cells in the uterine endometrium following neem treatment, indicating enhanced antigen-presenting ability of the uterus; a feature that may be related to the observed antifertility effect of neem oil. The present investigation demonstrates that an IUNT can be used for long-term, reversible contraception, without any apparent side effects, and that the method could provide an alternate to currently used intrauterine contraceptive devices (IUCD) (*Upadhyay et al., 1994*).

Praneem polyherbal cream, a spermicidal formulation, has been developed at the National Institute of Immunology, which makes use of Praneem, a purified extract from the dried seeds of an ancient Indian plant *A. indica* (Neem), extract from the pericarp of fruits of Sapindus species and quinine hydrochloride. These ingredients have a synergistic spermicidal activity and an optimised formula was derived. The components were made into a watersoluble cream base prepared by using pharmaceutically acceptable base and stabilised by addition of IP grade antioxidant and preservatives. The cream is devoid of irritation and sensitization potential, as seen with standard Draize test on normal and abraded skin of rabbits and by 21-day cumulative skin sensitivity in human volunteers. The formulation was found to be safe under subacute toxicity studies in monkeys. The formulation has shown high contraceptive efficacy in rabbits and in monkeys after intravaginal application. The shelf-life of the cream at room temperature is estimated to be 18 months by accelerated stability studies. In India, the National Institute of Immunology has developed Praneem polyherbal cream as a vaginal spermicide. Scientists combined a purified extract from the dried seeds of an ancient Indian plant *A. indica*, extract from the pericarp of fruits of Sapindus species, and quinine hydrochloride with a pharmaceutically acceptable base to make a watersoluble cream base. They added IP grade antioxidant and preservatives to stabilize the cream base. They applied the cream on a shaved or abraded part of the skin of human volunteers and rabbits and inserted it into the vagina of Bonnet monkeys to test for sensitivity and irritation. They studied the dissolution characteristics of the cream after intravaginal application in the rabbits and monkeys. They compared pregnancy rates of monkeys who received intravaginal application of 2 ml cream every day with those of control monkeys. Praneem polyherbal cream did not irritate the skin of the rabbits or the human volunteers. The accelerated stability studies found the shelf-life of the cream at room temperature to be 18 months. The cream dissolves entirely within 30 minutes in the vaginal secretions of the

rabbits and 40 minutes in those of the monkeys. Precoital application of the cream provided complete protection against pregnancy in rabbits in the 1st 30 minutes after application. The conception rate was acceptable at 60 minutes (7%), but thereafter it climbed to unacceptable levels (28.6% at 90 minutes and 75% at 12 hours). The conception rate of monkeys who received precoital application of Praneem polyherbal cream was only 2.27%. These results suggest that Praneem polyherbal cream can protect against pregnancy without causing irritation. Its antimicrobial properties provide another advantage (*Garg et al., 1993*). In vitro evidence is presented showing toxicity of neem oil on sperm-egg interaction in mouse. Cumulus oophorus-enclosed ova, inseminated with capacitated spermatozoa, were cultured in 1 ml of in vitro fertilization (IVF) medium and over layered by 1 ml of different concentrations of neem oil (1, 5, 10, 25, 50 and 100%) for IVF duration of 4h. At the end of incubation, ova were allowed to grow in neem oil-free culture medium and assessed for fertilization, first cleavage (2-cell formation) and blastocyst formation in vitro at 4-14h, 24h and 108h postinsemination respectively. The study showed that the presence of neem oil at concentrations of 10, 25 and 50% caused inhibition of IVF in a dose-dependent manner. The toxic effect of exposure of 25 and 50% neem oil was further carried over to the first cleavage of the resulting fertilized ova and the toxic effect of 5, 10, 25 and 50% was carried over to the blastocyst formation from the resulting fertilized ova when grown in neem-oil free culture medium. A total of 94.1% inhibition of 2-cell formation and 100% inhibition of blastocyst formation from the inseminated ova was observed in 50 and 25% neem oil-treated groups respectively. Neem oil at 100% concentration caused 100% degeneration of ova at 1h of sperm-ova coculture. The study showed a direct toxic effect of neem oil on sperm-egg interaction in vitro and encourages research investigations of this herbal product as a pre-coital contraceptive (*Juneja and Williams. 1993*). An alternate approach to vasectomy for long-term male contraception following a single intravas application of a traditional plant (A. indica) product having immunomodulatory properties is described. Male Wistar rats of proven fertility were given a single dose (50 microliters) of neem oil in the lumen of the vas deferens on each side; control animals received the same volume of peanut oil. Animals were put on continuous mating 4 weeks after the treatment, with females of proven fertility. While the control animals impregnated the female partners, all males treated with neem oil remained infertile throughout the 8 months of observation period. Epididymal and vas histology were normal without any inflammatory changes or obstruction. The intra-vas administration of neem oil resulted in a block of spermatogenesis without affecting testosterone production; the seminiferous tubules, although reduced in diameter, appeared normal and contained mostly early spermatogenic cells. No antisperm antibody could be detected in the serum. Unilateral administration of neem oil in the vas resulted in a significant reduction of testicular size and spermatogenic block only on the side of application; the draining lymph node cells of the treated side also showed enhanced proliferative response to in vitro mitogen challenge. These results indicate that the testicular effects following intra-vas application of neem oil may possibly be mediated by a local immune mechanism (Upadhyay et al., 1993). The International Development Research Centre of the Government of Canada has (1974) doveloped contraceptive method using the purified extract of the neem tree called praneem. This extract injected into the uterus of rats and monkeys. Researchers hope it can be a safe and effective method for women to use during the 3 months when they receive their vaccine shots. It is also working on perfecting the delivery system of the vaccine, e.g., a biodegradable implant releasing the required dosage over 1 year (Newton. 1993). Immunomodulatory effects of neem oil were studied in mice. The animals were treated intraperitoneally (i.p.) with neem oil; control animals received the emulsifying agent with or without peanut oil. Peritoneal lavage, collected on subsequent days, showed a maximum number of leukocytic cells on day 3 following treatment

with neem oil; peritoneal macrophages exhibited enhanced phagocytic activity and expression of MHC class-II antigens. Neem oil treatment also induced the production of γ-interferon. Spleen cells of neem oiltreated animals showed a significantly higher lymphocyte proliferative response to in vitro challenge with Con A or tetanus toxoid (TT) than that of the controls. Pre-treatment with neem oil, however, did not augment the anti-TT antibody response. The results of this study indicate that neem oil acts as a non-specific immunostimulant and that it selectively activates the cell mediated immune (CMI) mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge (*Upadhyay et al., 1992*). Neem oil, extracted from the seeds of *A. indica* has been found to act as a good spermicidal agent. Pre and post coital application of the oil intravaginally prevented pregnancy in rhesus monkey (Bardhan et al., 1991). NIM-76, the odorous and volatile fraction of neem oil, was investigated for its antifertility activity *in-vivo* in rats, rabbits and rhesus monkeys. The drug is effective when applied before coitus but not so when applied during post-coital stages. It, therefore, appears to act mainly by its spermicidal effect. No alteration in the estradiol and progesterone values was observed after the application of the drug in monkeys (*Riar et al., 1991*). Although barrier contraceptives were among the first methods of preventing unwanted pregnancy ever described for human use, with the advent of the non-coitally related oral contraceptives and intrauterine devices, they gradually fell into relative disuse. However, for a variety of reasons, this is no longer the case. There is a renewed interest in these techniques both as a major form of birth control and also as our best protection against the transmission of sexually transmitted diseases (STDs), many of which are now occurring in epidemic form. This latter reason has stimulated fresh approaches to both physical barriers and spermicidal agents. In addition, attempts have also been made to assess the true effectiveness of periodic abstinence and ways in which to make its use more accurate and acceptable. Concerns about preventing the transmission of STDs as well as pregnancy have led to a renewed interest in barrier contraception and spermicides. Although the condom has received greatest emphasis, data suggest that the use of a female barrier method such as the diaphragm and sponge may be even more effective; for adolescent females, at highest risk, combined use of barrier and oral contraception may be indicated. Given the documented low acceptability of the condom among groups at greatest risk and a lack of knowledge about its proper use, interest has focused on female condoms that cover the entire vagina, cervix, part of the female perineum, and the base of the penis. Spermicides enhance the effectiveness of barrier contraceptives, and new approaches--including use of neem oil and in vitro cobaltous ions-are under development. Finally, research is underway to enhance the effectiveness of various forms of periodic abstinence and ovulation detection techniques. Promising appears to be the supplemental use of a barrier method at the time of presumed ovulation (*Connell. 1991*).

A novel use of *A. indica* oil, a traditional plant product, for long-term and reversible blocking of fertility after a single intrauterine application is described. Female Wistar rats of proven fertility were given a single dose (100 microliters) of neem oil by intrauterine route; control animals received the same volume of peanut oil. Whereas all control animals became pregnant and delivered normal litters, the rats treated with neem oil remained infertile for variable periods ranging from 107 to 180 days even after repeated matings with males of proven fertility. The block in fertility was, however, reversible as half of the animals regained fertility and delivered normal litters by five months after treatment, without any apparent teratogenic effects. Unilateral administration of neem oil in the uterus blocked pregnancy only on the side of application whereas the contralateral uterine horn treated with peanut oil had normally developing foetuses; no sign of implantation or foetal resorption was noted in the neem-oil-treated horn. The ovaries on both sides had 4-6 corpora lutea indicating no effect of treatment on ovarian functions. The

animals treated with neem oil showed a significant leukocytic infiltration in the uterine epithelium between days 3 and 5 post coitum, i.e. during the pre-implantation period. Intrauterine application of neem oil appears to induce a pre-implantation block in fertility; the possible mechanisms of the antifertility action are discussed (*Upadhyay et al., 1990*). The volatile, odorous steam distillation fraction of neem oil (NIM-76) exhibited *in-vitro* spermicidal activity. The minimum concentration which inhibited spermatozoal motility was 0.25mg/ml for rat and 25mg/ml for human spermatozoa. The effect of the extract on spermatozoal motility was found to be dose-dependent. The activity of extract was not altered in the presence of vaginal or cervical mucus. Intra-vaginal application of NIM-76 in rabbits showed no irritation to the vaginal mucosa (Riar et al., 1990). The antifertility effect of the antiestrogenic substance neem oil, extracted from the seeds of *A. indica*, acts directly on the uterus or through absorption from the vaginal epithelium into the general circulation. 25µl neem oil was administered intravaginally. The study showed that the neem oil exerts its effect on the endometrium through absorption into the general circulation from the vaginal epithelium. The antiestrogenic quality of neem oil explains its anti-implantation effect. But the post implantation effect, which caused implanted fetuses to be either resorbed or expelled, may be due to direct toxicity, to a fall in progesterone level, or to interference with the uterine utilization of progesterone (*Riar et al., 1988*). Neem oil, a natural product of *A. indica* was investigated for various hormonal properties in relation to its post-coital contraceptive action. At subcutaneous doses up to 0.3 ml/rat, neem oil did not possess any estrogenic, anti-estrogenic or progestational activity and appeared not to interfere with the action of progesterone. These findings were confirmed using the histo-architecture of the uterus of treated rats. Since the post-coital contraceptive effect of neem oil seems to be non-hormonal, neem oil would be expected to elicit less side effects than the steroidal contraceptives (*Prakash et al., 1988*).

Discussion: Although barrier contraceptives were among the first methods of preventing unwanted pregnancy ever described for human use, with the advent of the non-coitally related oral contraceptives and intrauterine devices, they gradually fell into relative disuse. However, for a variety of reasons, this is no longer the case. There is a renewed interest in these techniques both as a major form of birth control and also as our best protection against the transmission of sexually transmitted diseases, many of which are now occurring in epidemic form. This latter reason has stimulated fresh approaches to both physical barriers and spermicidal agents. In addition, attempts have also been made to assess the true effectiveness of periodic abstinence and ways in which to make its use more accurate and acceptable. Concerns about preventing the transmission of sexually transmitted diseases as well as pregnancy have led to a renewed interest in barrier contraception and spermicides. Although the condom has received greatest emphasis, data suggest that the use of a female barrier method such as the diaphragm and sponge may be even more effective; for adolescent females, at highest risk, combined use of barrier and oral contraception may be indicated. Given the documented low acceptability of the condom among groups at greatest risk and a lack of knowledge about its proper use, interest has focused on female condoms that cover the entire vagina, cervix, part of the female perineum, and the base of the penis. Spermicides enhance the effectiveness of barrier contraceptives, and new approaches--including use of neem oil and in vitro cobaltous ions-are under development. Finally, research is underway to enhance the effectiveness of various forms of periodic abstinence and ovulation detection techniques. Promising appears to be the supplemental use of a barrier method at the time of presumed ovulation (*Connell. 1991*). From the perspective of developing countries - or any woman concerned about the long-term impact of using hormones for birth control-finding a method of contraception that is effective, inexpensive and easily available is truly a step toward solving global problems.

2. Conclusion:

Spermicide is a contraceptive substance that eradicates sperm, inserted vaginally prior to intercourse to prevent pregnancy. As a contraceptive, spermicide may be used alone. However, the pregnancy rate experienced by couples using only spermicide is higher than that of couples using other methods. Usually, spermicides are combined with contraceptive barrier methods such as diaphragms, condoms, cervical caps, and sponges. Combined methods are believed to result in lower pregnancy rates than either method alone. Spermicides are unscented, clear, unflavored, non-staining, and lubricative. Spermicides cause irritation and according to the CDC, studies have shown that spermicides increase the risk of HIV. Contraceptive Technology states that spermicides have a failure rate of 18% per year when used correctly and consistently, and 29% under typical use. Extractives of the neem plant such as neem oil have also been proposed as spermicides based on laboratory studies. Animal studies of creams and pessaries derived from neem have shown they have contraceptive effects, however trials in humans to determine its effectiveness in preventing pregnancy have not yet been conducted. Spermicides are believed to increase the contraceptive efficacy of condoms. However, condoms that are spermicidally lubricated by the manufacturer have a shorter shelf life [18] and may cause urinary-tract infections in women. The WHO says that spermicidally lubricated condoms should no longer be promoted. However, they recommend using a nonoxynol-9 lubricated condom over no condom at all.

3. References:

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