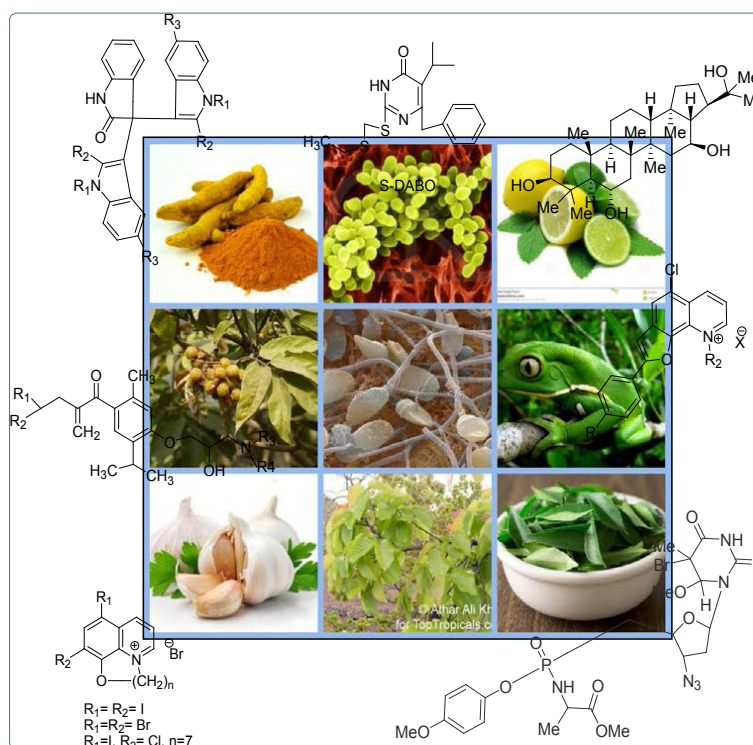**Review Article**

Insights of Spermicidal Research: An Update

Maitreyee Banerjee, Abhijit Hazra, Yogesh P Bharitkar and Nirup B Mondal*

Chemistry Division, Indian Institute of Chemical Biology, Council of scientific & Industrial Research,4 Raja S. C. Mullick Road, Jadavpur, Kolkata- 700 032, India

Abstract

Spermicides came into existence as a method of contraception for over hundred years. Since then, investigations and developments in this field have led to more and more fruitful as well as promising results but still left enough scope for further studies. The titled article manifests a brief discussion on the development of spermicides from different natural and synthetic sources, a compilation of the promising molecules and even extracts and includes an outline of the basic physiology behind the bioactivity. Since spermicides are only one among several other methods of contraception adopted and practiced, an attempt has been made to probe the necessity and advantage of spermicides over other modes of contraception, emphasizing on the requirements and scope of future investigations in this field.

Introduction

The growing population is a global problem with grave implications for the future. A report of the United Nations dated October 2011 reveals that the earth's population has passed seven billion [1]. The figure is double of that (3.5 billion) in 1968 and is estimated to increase by two more billions by the year 2050 [1]. Importantly, more than 90% of the world's future population growth is projected to take place in the developing world, where countries are already struggling to provide their people with food, water, health care, education and jobs [2]. Moreover, nearly 215 million women in the developing world want, but do not have access to, modern contraception, which leads to unintended pregnancies, unsafe abortions, maternal and child mortality and morbidity, reduced access to education and a whole range of other negative consequences [3]. Unintended pregnancy is a fundamental problem asking for better, more accessible methods of contraception, and constitutes a fundamental public health need in today's world. Nearly 60 percent of all pregnancies in the United States and anywhere from 24 to 64 percent of all pregnancies worldwide are unintended, either because they were mistimed or because they were not wanted

***Corresponding author:** Nirup B Mondal, Chemistry Division, Indian Institute of Chemical Biology, Council of scientific & Industrial Research,4 Raja S. C. Mullick Road, Jadavpur, Kolkata- 700 032, India, Tel: +91 33 2499 5721; Fax: +91 33 2473 5197; E-mail: nirup@iicb.res.in

at all [4]. Effective family planning facilities and newer contraceptive modalities are in great demand to combat this alarming situation. It is now generally accepted that the currently available methods of fertility regulation are inadequate to meet the varied and changing personal needs of couples at different times in their reproductive lives. The available contraceptive options are limited in their scope to respond to variability in individual and family situations, to cultural differences, to specific health problems and shifting personal preferences, and to life cycle stages and changing reproductive intentions across those stages. Although various physical and chemical methods apart from male and female sterilization have been in use to prevent unwanted pregnancies, none of the approaches can be considered as ideal. Though oral contraceptive pills are preferred by gynecologists as the safest drugs for preventing unwanted pregnancies, they fail to give protection against STIs. The barrier methods of contraception presently available, e.g., diaphragm, cervical cap, contraceptive sponge and spermicides, appear to put the users at higher risk of vaginal infection, urinary tract infection and possibly toxic shock syndrome. Vaginal contraceptive products that have been available for many years usually contain the membrane surfactant nonoxynol-9 (N-9) as one of the main ingredients [5]. However, the major drawback of using N-9 or other surfactants lies in their detergent-type cytotoxic effect on vaginal cells [6]. Besides, N-9 is also known to inactivate *Lactobacilli*, which form the normal flora in vaginal tissues [7]. Disturbance of the vaginal micro flora increases the vulnerability to vaginal infections, which in turn enhances the chances of STI/HIV transmission [8]. Hence, in the present global scenario with alarming population growth and increasing threats of AIDS and other STDs, development of vaginal spermicidal microbicides lacking detergent-type cytotoxicity may offer a significant clinical advantage over the currently marketed spermicides. We therefore decided to review the history of research in the field of vaginal contraceptives with special emphasis on the development of safer, more effective spermicides as summarized in this review.

Physiological Basis of Barrier Contraception

Fertilization in mammals is a multi-step process that terminates in the fusion of the sperm and oocyte plasma membrane in the oviduct. During the menstrual cycle, mature egg released from the ovarian follicle enters and begins to travel through the fallopian tube and remains viable in the oviduct for about 24 hr. At ejaculation, sperm is deposited into the vagina and must overcome a series of barriers in their journey from vagina to oviduct, each of which eliminates a substantial proportion of the original population of sperm. It will live for an average of three days in the female reproductive tract and for successful fertilization, live and motile sperm has to reach the oviduct within this time. Sperm must locate and penetrate the awaiting egg while it is in the fallopian tube. Post-epididymal spermatozoa are motile cells, and sperm motility becomes critical at the time of fertilization because it allows or at least facilitates the passage of the sperm through the isthmus part of the oviduct at the ipsilateral side where ovulation takes place [9-11].

The basis of barrier methods of contraception is to prevent entry of sperm into the endo-cervical region of the reproductive tract. This can be achieved either by putting a mechanical barrier, or a chemical barrier that may render the cervico-vaginal milieu unfavorable for sperm survival or transport, thereby blocking the successful union of gametes. Also, the barrier methods are a good choice for couples that need a back-up method while waiting for another method to take effect, or after using oral contraceptives inconsistently, or having intercourse infrequently.

Barrier methods of contraception can be classified into three major categories as follows:

- Mechanical barrier: includes the male condom, female condom, diaphragm, cervical cap, and sponge.

- Chemical barrier or spermicides: Spermicides are agents that kill spermatozoa or render them incapable of normal function. A spermicide may act at or near the exocervix to kill spermatozoa before they enter the upper female genital tract. They are available in topical formulations as foams, creams, gels, films or suppositories, which are often used for contraception in conjunction with mechanical barriers and other devices.

In contrast to agents that must be taken orally or implanted in the body, vaginal contraceptives are known as coitus related or episodic products; in that they only need to be used when sexual activity is imminent. Thus, females are not exposed to the agent through the month, as they are with oral contraceptives, intrauterine devices, implanted inserts or depot injections. They are free from side effects of systemic exposure to the contraceptive agents. The barriers are the only devices that may afford some protection during a sudden necessity. Moreover, breastfeeding women who need contraception may use spermicides since these preparations usually increase vaginal lubrication, can be used immediately after childbirth, and have no hormonal side effects. These methods may be suggested for women over 40 and those with contraindications to oral contraceptives and IUDs (intrauterine devices)

- Combined physicochemical barrier: The spermicidal agents, when used alone, provide some contraceptive protection, but are best when used with another barrier method. This forms the basis of developing combined physicochemical barrier method of contraception. The effectiveness of barrier methods may be increased when two or more options are combined together. For example, latex condoms are available with a coat of spermicide. Use of dual protection is particularly attractive when there is a need to reduce the risk of sexually transmitted infections (STIs) including human immunodeficiency virus (HIV) along with preventing unwanted pregnancy. Multiple methods to eliminate STDs and improve contraceptive effectiveness, particularly where one or more methods is controlled by the woman, will increase consistent use of at least one.

Spermicides as the Method of Barrier Contraception

Spermicides as discussed earlier are a biologically effective way of interrupting fertility and have the advantage that they do not depend on highly skilled personnel for their prescription and use. Spermicidal agents are defined as drugs that have the ability to immobilize or kill the sperm upon contact. An ideal spermicide should immediately and irreversibly produce immobilization of the sperm, be nonirritating to the vaginal and penile mucosa, not have adverse effects on the developing fetus, be free from long term topical and systemic toxicity, and should not be systemically absorbed. Hence, the spermicidal agents under development should be critically evaluated on these aspects.

Chemical Classifications of Spermicides

Spermicides can be broadly classified into the following major divisions based on their origin:

Natural spermicides

This class includes all the spermicidal agents derived from natural sources like plants, microbes and animals. The isolated and identified compounds as well as extracts with established specific spermicidal properties are discussed below. Medicinal plants have been screened

for decades in search of spermicidal compound/s. The compounds responsible for the spermicidal activity may act singly or through a synergistic action.

Gossypol: A di-sesquiterpene aldehyde, isolated from the seeds of cotton (*Gossypium* species) plant, is reported to be a spermicidal agent [12]. The MEC (minimum effective concentration) is estimated to be 40 mg/mL [13]. Gossypol (Figure 1) inhibits sperm motility by blocking ATP production and utilization. It acts on mitochondria, suppressing oxygen consumption, inhibiting the pyruvate dehydrogenase and ATPase activities, and probably acts on the motility apparatus both by blocking dynein ATPase activity and preventing protein phosphorylation [14].

Hederagenin derivatives: Saponins isolated from *Sapindus mukorosii* (*reetha*) have shown most potent spermicidal activity. The saponins reported are derivatives of hederagenin (Figure 2) namely mukurozi-saponins E1, G, X, Y1, Y2, Z1 and Z2 [15]. After incubation with saponins at 0.5 mg/mL for 1 min, the spermatozoa did not exhibit any significant morphological changes, though at the same concentration, immobilization of the sperms was observed. At higher concentrations of saponins (1-50 mg/mL) spermatozoa displayed marked disruption, vacuolation, vesiculation and erosion of the membrane covering the head region. Coiling of the tail was also noticeable with higher concentrations of saponins though no damage was evident under SEM in the flagellar region of the sperm [16,17]. These saponins were formulated into a contraceptive cream named 'CONSAP'. This cream has completed Phase III clinical trials successfully in India [18].

Allitridium: Allitridium (Figure 3) isolated from garlic (*Allium sativum*) showed complete immobilization of sperms from human and animals within 20 sec at 7.5 mg/mL and within 3 min at 1.5 mg/mL. At the dose of 7.5 mg/mL, it showed no vaginal irritation reaction or other side effects. It had no bacteriostatic action on the lactic acid bacilli, so is
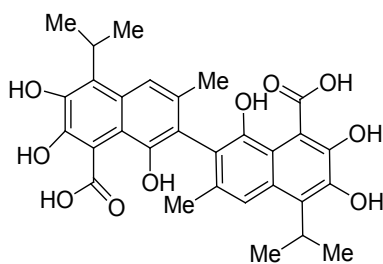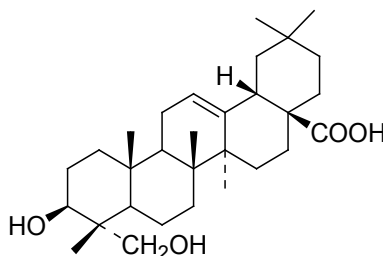

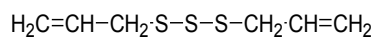**Figure 1:** Gossypol.

Figure 2: Hederagnin.

$$H_2C=CH-CH_2 \cdot S-S-S-CH_2 \cdot CH=CH_2$$
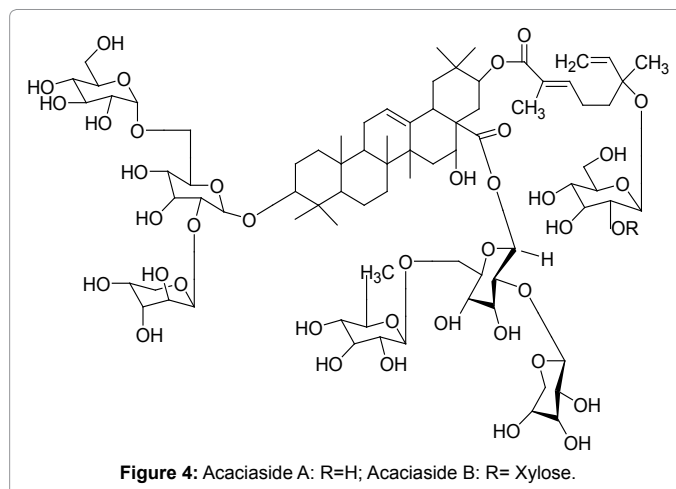
Figure 3: Allitridium.

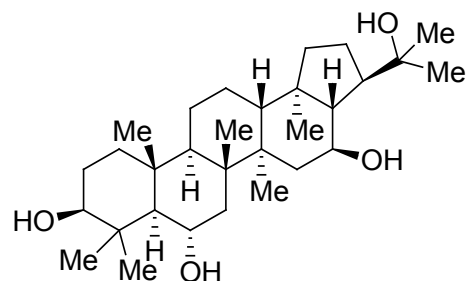**Figure 4:** Acaciaside A: R=H; Acaciaside B: R= Xylose.

Figure 5: Mollugogenol A.

unlikely to interfere with the growth of the bacilli in the vagina [19].

Acaciasides A and B: Mixtures of two difficult to separate triterpenoid saponins isolated from the powdered seeds of *Acacia auriculiformis* have shown spermicidal activity at the concentration of 0.35 mg/mL. The aglycone parts of these two saponins were characterized as acacic acid lactone and the monosaccharide constituents were identified as D-glucose, D-xylose, L-arabinose, and L-rhamnose. The complete chemical structures of these compounds are given in the Figure 4. Regarding their mode of action, electron microscopic observation showed that the plasma membrane was disintegrated and total dissolution of the acrosomal cap took place [20,21].

Mollugogenol A: The ethyl acetate fraction of *Mollugo pentaphylla*, a tropical herb, contains an antifungal triterpenoid saponin mollugogenol-A (Figure 5) which has demonstrated spermicidal activity at 300 µg/mL. Electronic microscopic observation showed that fragmentation or loss of plasma membrane, vesiculation of periacrosomal membrane and dissolution of the organelle took place, suggestive of sperm degeneration [22].

Salannin: A limonoid bitter principle of the seed oil of *Azadirachta indica*, it (Figure 6) showed antiulcer, antibacterial and spermicidal activities [23].

Curcumin: Curcumin (diferuloyl methane), a yellow pigment present in the rhizomes of turmeric *(Curcuma longa)* and related species used as spice, has a wide array of pharmacological and biological activities. Studies have demonstrated that curcumin (Figure 7) has anti-tumor, anti-inflammatory and anti-infective activities. It also inhibits the integrase activity of the HIV. When curcumin is used in the concentrations of 30 µg/mL and 100 µg/mL, the human sperm motility was reduced to 53.4% and 4.1% after 120 min. Full immobilization was
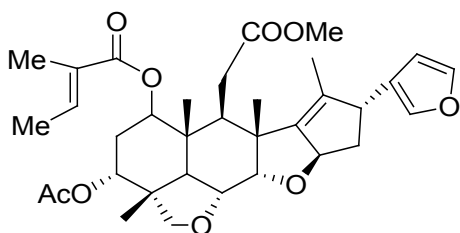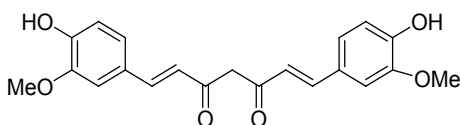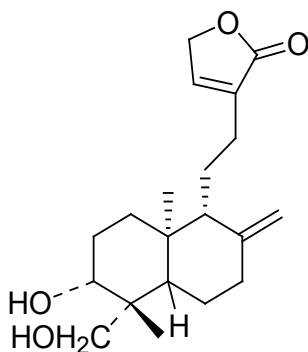
Figure 6: Salannin.

Figure 7: Curcumin.

Figure 8: 14-deoxyandrographolide.

Oleanolic acid 3-β-D-glucuronide: Oleanolic acid 3-β-D-glucuronide (OAG) is an active principle isolated from root extracts of *Sesbania sesban* has been evaluated for its spermicidal activity by [27] and the spermicidal MEC was found to be 50 μg/mL. TEM and LPO revealed that OAG affected the sperm membrane integrity. OAG (Figure 10) declined fertility to zero, was nonmutagenic and was not harmful to *Lactobacillus*.

Asiatic acid: Asiatic acid (Figure 11) isolated from *Shorea robusta,* abundantly available in the forests of Southeast Asian countries and cultivated for its strong timber throughout the Indian subcontinent, possesses appreciable spermicidal and microbicidal potential [28].

Desgalactotigonin (DGT): Crude decoction of *Chenopodium album* seed showed spermicidal effect at MIC 2 mg/ml in earlier studies. Systematic isolation, characterization and evaluation revealed that the major metabolite Desgalactotigonin (DGT) (Figure 12) is the most effective principle in both *in vitro* and *in vivo* studies. The minimum

Figure 9: Mi-Saponin A.

achieved with a dose of 300 μg/mL at the end of 60 min. Curcumin-induced change in sperm mitochondrial transmembrane potential indicate that this compound may interfere with sperm energy metabolism. Used in micro molar concentrations, it inhibits protein kinase C, which is present in human sperm and is believed to play a role in modulating human sperm flagellar movement [24].

14-Deoxy andrographolide: *Andrographis paniculata* is a small herb abundantly available in South East Asia particularly in Indian sub-continent and China, and is one of the commercially exploited traditional medicinal plants used for various ailments. 14-Deoxy Andrographolide (Figure 8), a labdane diterpene isolated from this plant, has been found to possess significant spermicidal potential [25]. The MEC of 14-deoxyandrographolide was found to be 200 μg/ml which was lower than standard spermicide N-9.

Mi-Saponin A: The seed extracts of *Madhuca latifolia* were reported to have spermicidal activity. Mi-saponin A (MSA) was found to be the most potent component among a mixture of saponins (Figure 9). The mean effective concentration of MSA that induced irreversible immobilization was 320 mg/mL for rat and 500 mg/mL for human sperm. The spermicidal effect involved increased membrane LPO leading to structural and functional disintegration of sperm plasma membrane and acrosomal vesicle. A comparative in vitro cytotoxicity study in human vaginal keratocyte (Vk2/E6E7) and endocervical (End/E6E7) cell lines demonstrated that the 50% cell cytotoxicity (CC_{50}) values, and consequently the safety indices, for MSA were ≥8-fold higher as compared to those of N-9 [26].
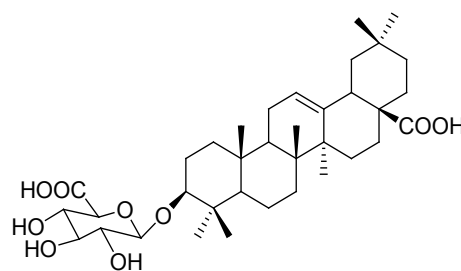

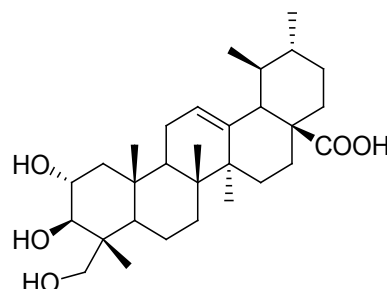**Figure 10:** Oleanolic acid 3-β-D-glucuronide.
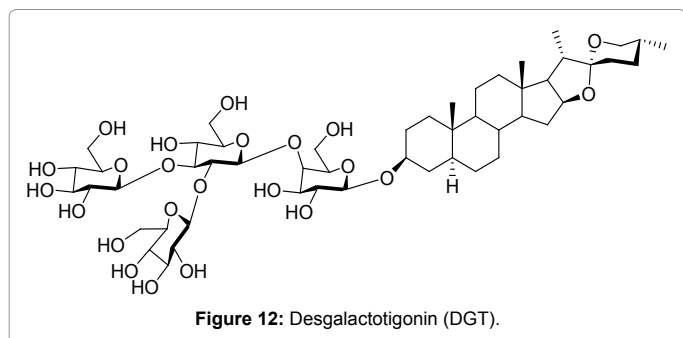

Figure 11: Asiatic acid.

Figure 12: Desgalactotigonin (DGT).

effective concentration (MEC) of DGT that induced instantaneous immobilization in vitro was 24.18 mM for rat and 58.03 mM for human spermatozoa. Implantation was prevented in DGT treated uterine horn while no hindrance occurred in the untreated contra lateral side. Desgalactotigonin emerged 80 and 26104 times more potent than the decoction and Nonoxynol-9 respectively. It possesses mechanism based detrimental action on both human and rat spermatozoa and spares lactobacilli and HeLa cells at MEC which proves its potential as a superior ingredient for the formulation of a contraceptive safer/compatible to vaginal microflora [29].

Plant extracts containing principles with spermicidal activities

Chenopodium album: Aqueous decoction of seeds of *Chenopodium album*, a small herb that grows all over India as a common agricultural weed, has been found to possess contraceptive efficacy in laboratory mammals [30]. The *Chenopodium album* decoction (CAD) was found to be an intimate mixture of two glycosides. Further studies indicated that both glycosides have the common genin, oleanolic acid, but differ in the interglycosidic linkage/aglycon–sugar linkage. The complete chemical structure (sugar-sugar and sugar-aglycon linkage) of the glycosides is yet to be established. The MEC for 100% immobilization of sperm was found to be 2 mg/mL. Sperm death mediated by CA extract was found to be due to oxidative damage of cellular macromolecules by in situ generation of ROS (reactive oxygen species) [31]. The safety standard evaluation for CA extract strengthens the candidature of CAD as a safe microbicidal spermicide. It is almost non-irritant to rabbit skin and rat vaginal tissues at doses 10 fold higher than its hemolytic index. However, CAD did not block the growth of Lactobacillus, although the size of individual colony was marginally reduced. It prevented the growth of the fungal pathogen *Candida albicans* at 20 mg/ml of CAD. [32]

Other plants: Other plants containing saponins, which have shown spermicidal activity, include *Phytolacca dodecandra*, *Calendula officinalis*, *Acacia cassia*, *Acacia concinna*, *Trigonella foenum-graecum* [33], and *Cestrum parqui* [34]. Saponins are naturally occurring and there is no report of their systemic toxicity. However, due to their capacity to reduce interfacial tension, they may alter the permeability of the vaginal membrane on frequent use. In addition, reduction in interfacial tension may, in fact, lead to decreased viscosity of the mucus and hence result in an increased rate of transfer of spermatozoa through the vaginal mucosa. Therefore, it is necessary to critically evaluate these effects before advocating the use of saponins as spermicidal agents [35].

Neem extracts: The neem tree, *Azadirachta indica*, is indigenous to the Indian subcontinent. Neem oil, 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 [36]. Khillare has revealed that 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 (Table 1) [37].

Praneem polyherbal formulations: A combination formulation developed as "Praneem polyherbal cream", which includes a purified extract from the dried seeds of *Azadirachta indica* (250 mg/mL), extract from the pericarp of fruits of *Sapindus mukorosii* (0.5 mg/mL) and quinine hydrochloride (3.46 mg/mL), has shown spermicidal activity in 20 sec. The formulation has shown high contraceptive efficacy in rabbits and in monkeys after intravaginal application. Also the formulation was found to be safe with no vaginal irritation when applied intravaginally for 30 days at a daily dose of 1 mL [38]. The Praneem polyherbal pessary and tablet formulated includes purified ingredients from neem leaves, *Sapindus mukorosii* and *Mentha citrata* oil. The vaginal pessary has shown potent spermicidal action of human spermatozoa in vitro and high contraceptive efficacy was demonstrated in rabbits of proven fertility [39,40]. Praneem polyherbal formulations have shown in vitro activity against HIV and sexually transmitted disease pathogens [40]. Praneem vaginal pessaries and tablets were found to be safe for once daily intravaginal use consecutively for 7 and 14 days in healthy women volunteers [41,42]

Spermicides from natural sources other than plants

Dermaseptins and magainins: These are two classes of cationic, amphipathic α-helical peptides that have been identified in the skin extracts of frogs and *Xenopus laevis* [64]. Magainins A and G, which are two natural peptides having 23 amino acids and differ by only two substitutions, have been found to have a wide spectrum in vitro antimicrobial activity against gram positive and negative bacteria, fungi, and protozoa. Considering their contraceptive activities and their effect against various STI-causing pathogens, it is believed that these two peptides are appropriate candidates for the evaluation of newer and safer microbicides spermicides in the future [65]. Magainin-A was found to be more potent than magainin-G. Intravaginal administration of magainin-A 200 μg to rats [66] and 1 mg to rabbits and monkeys once before mating resulted in 100% sperm immobilization [67,68]. Magainin-A does not have overt cytotoxic properties and is safe for intravaginal application. It is also active against various STI-causing pathogens but not against HIV-1 and HIV-2. It is reported that effectiveness of magainin as a contraceptive *in vivo* is possibly due in part to the removal of cholesterol from sperm membranes [69].

Microorganisms: Microorganisms are also known to inhibit sperm motility either directly by agglutinating the spermatozoa or excreting extracellular products that immobilize the sperms.

Nisin: It is a 34 amino acid, naturally occurring antimicrobial cationic peptide known to be produced by the bacteria *Lactococcus lactis*. Nisin has been used as a food preservative throughout the world and the World Health Organization (WHO) and US, Food & Drug Administration have conferred GRAS status to this peptide. At the dose of 300-400 μg, complete immobilization of human spermatozoa was observed within 20 sec. *In vivo* contraceptive efficacy studies in rats showed complete arrest of sperm motility and no pregnancy in any of the animals. At the contraceptive dose of 200 μg, Nisin did not alter the morphology of the vaginal epithelial cells, nor did it cause any

Sr. no	Name of the plant	Parts used	Types of plants extract/ active principle	Animal model	Activities	Ref.
1	*Acacia concinna DC*	Stem bark	Acacic acid	Rat	Spermicidal and semen coagulation activities	43
2	*Acacia auriculaeformis A. Cunn.*		Triterpene saponins		Sperm immobilizing effect	20
3	*Acacia cassia* Wight & Arn.	Fruit	Saponins		Sperm immobilizing effect	44
4	*Achyranthes aspera Linn.*	Root	50% ethanol extract	Rat	Spermicidal action	45
5	*Albizia procera (Roxb.) Benth*	Seed, root	Proceric acid and leanic acid	Rat	Spermicidal and semen coagulation activities	43
6	*Anagallis arvensis Linn*	Whole plant		Rat	Spermicidal and semen coagulation activities	43
7	*Azadirachta indica A.Juss*	Seed	Seed oil	Rat	Spermicidal activity	46
			Neem oil	Monkey, Human	Spermicidal activity	47
		Leaves	Aqueous extract	Rat	Spermicidal activity	37
8	*Bursera Sp*	Stem, leaf	Saponins, leaf extract	Human, Bovine	Sperm aggregation	48
9	*Carica papaya Linn*	Seed	chromatographic sub fraction of chloroform extract	Human	Spermicidal activity	49
10	*Citrullus colocynthis Schrad*	Fruit	50% ethanol extract	Rat	Impairment of sperm	50
11	*Cyclamen persicum Mill.*	Whole plant	Saponins		Spermicidal activity	51
12	*Diploclisia eshinatus Linn*.	Stem	Ecdysterone		Spermicidal activity	52
13	*Eshinops eshinatus Roxb*	Root	50% ethanol extract	Rat	Sperm antimotility. reduces sperm density in cauda epididymis	53-54
14	*Embelia ribes Burm.f.*	Berry	Embelin	Rat	Spermicidal activity	55
15	*Eupatorium brevipes* DC.		Brevipenin		Spermicidal activity	56
16	*Hedera nepalensis* K.Koch	Inflorescence			Immobilization of spermatozoa	57
17	*Justicia simplex* D.Don	Flower	Justicisa saponin		Sperm acrosomal membrane stabilizing action	58
18	*Melodinus fusiformis* Champ. Ex Benth.		Solasodine (steroidal alkaloid)		Spermicidal activity	59
19	*Ophiopogon intermedius* (D,Don) Maxim	Rhizome	n-octacosanal, β-sitosterol		Spermicidal activity	60
20	*Pittosporum neelgherrense* Wight &Arn	Plant without root	Pittosida-A and Pittosida-B		Spermicidal and semen coagulation activities	61
21	*Primula vulgaris* Huds.		Saponins		Immobilization of spermatozoa	62
22	*Pyrus cuspidata Betrol.*	Plant without root	Extract		Spermicidal and semen coagulation activity	43
23	Annona squamosa	Seeds	Hydroalcoholic extract	human	Spermicidal activity	63

Table1: Summery of extracts of different medicinal plants with spermicidal activity (1980-2005).

histopathological lesions in the vaginal epithelium when administered intravaginally for 14 consecutive days. The mechanism by which Nisin exerts its rapid spermicidal action is not known. However, the existing evidence suggests that it possesses an overall positive charge and interacts preferentially with anionic phospholipids. The sperm plasma membrane contains high concentration of phosphatidylglycerol, a strong anionic phospholipid moiety, and thus Nisin may have high affinity towards spermatozoa [70].

Sophorolipids: Sophorolipids (SLs) are a group of microbial glycolipids produced by yeasts, such as *Candida bombicola*, *Yarrowia lipolytica*, *Candida apicola,* and *Candida bogoriensis* [71]. Various forms of sphorolipids produced by *Candida bombicola* showed spermicidal and virucidal activities. They occur naturally as the disaccharide sophorose linked glycosidically to the hydroxyl group at the penultimate carbon of primarily C18-chain-length fatty acids [72]. The sophorolipid diacetate ethyl ester derivative (Figure 13A) is the most potent spermicidal and virucidal agent of the series of SLs studied. Its virucidal activity against HIV and sperm-immobilizing activity against human semen are similar to those of nonoxynol-9. However, it also induced enough vaginal cell toxicity to raise concerns about its applicability for long-term microbicidal contraception. Its structure-activity relationship has been established for creating new analogs with less cytotoxicity and higher activity.

Further studies conducted by Doncel et al. [73] revealed that sophorolipid analogues prepared by amino acids conjugated by forming amide bonds between their R-amino moiety and the carboxyl group of ring-opened SL fatty acids (Figure 13B) resulted in a new series with improved activities.

The influence of different uropathogenic microorganisms such as *Escherichia coli, Enterococcus, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Candida albicans* on human sperm motility has been studied in vitro by Huwe et al. [74]. Yin et al. showed that at high concentrations, *Streptococcus viridians* could induce sperm agglutination in vitro [75]. Jiang and Lu reported that *Staphylococcus aureus* significantly immobilizes the spermatozoa [76].
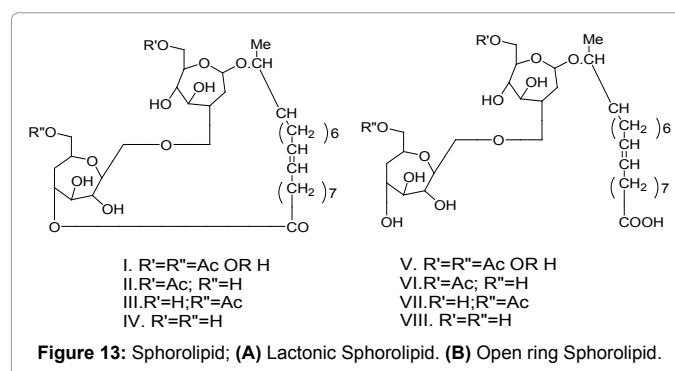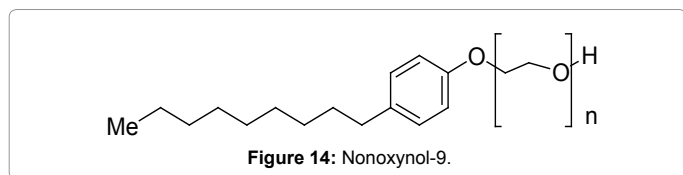

Figure 13: Sphorolipid; **(A)** Lactonic Sphorolipid. **(B)** Open ring Sphorolipid.

Figure 14: Nonoxynol-9.

In 2005 Ohri et al. was successful in isolating of spermatozoal agglutinating factor was isolated from *Staphylococcus aureus* isolated from a woman suffering from unexplained infertility [77]. The contraceptive efficacy of SAF was further evaluated using mouse as an *in vivo* model by the same group. The results indicated that 2.5 micrograms of SAF was sufficient to arrest sperm motility and protect against pregnancy [78].

Synthetic spermicides

These include the synthetically derived compounds which have been proved to possess spermicidal properties. In several of the cases significant anti-microbial activities are also found and the products can be categorized as dual action spermicidal microbicide. The different classes of synthetic compounds which have shown spermicidal effects and are being used as spermicides or may be used in future are discussed below.

Non-ionic surfactants: In the category of non-ionic surfactants octoxynol and nonoxynol are the two commonly reported surfactants. Nonoxynol is more potent spermicide than octoxynol. Among the different nonoxynol derivatives designated as N1-N15, p-nonyl phenoxy polyethoxy ethanol (N-9) is reported to be the most potent spermicide [79]. At present N-9 (Figure 14) is the only marketed contact spermicide available worldwide as gel, cream, foam and pessary formulations in various strengths. At the dose of 500 μg/ mL N-9 completely abolishes all sperm movement within one minute of addition. Although, N-9 has been employed as a contact spermicide for the last 30 years and is well tolerated, reports have appeared suggesting that the frequent use of surface active spermicides can be associated with vaginal irritation and the appearance of lesions in the epithelium. It has also been shown that nonoxynol-9 kills the natural vaginal flora (*Lactobacillus*) [80-83] causing disturbance of the normal (acidic) vaginal pH and weakening the natural protection against STIs, reproductive-tract infection (RTIs) and urinary-tract infections (UTIs) [84].

More recent studies report that the use of N-9 as a microbicide or in limiting the transmission of STD's should be discontinued since it interferes with the lipid bilayer of the vaginal epithelium and facilitates the process of absorption and transmission of the viral particle into the blood stream [85-88].

Ahmad et al. have designed a new bioadhesive suppository of N-9 called Long Acting, Sustained Release Spermicide (LASRS) that possesses advantages over the presently marketed formulations by having long-term efficacy and by forming a bioadhesive, protective layer over the genital tract epithelium [89,90].

Zidovudine derivatives: Zidovudine, i.e. 3'-azido-3'-deoxythymidine lacks in having spermicidal activity by itself, but two of its novel phenyl phosphate derivatives WHI-05 [5-bromo-6-methoxy-5,6-dihydro-3'-azidothymidine-5'-(p-methoxy-phenyl) methoxyalaninyl phosphate] (Figure 15A) and WHI-07 [5-bromo-6-methoxy-5,6-dihydro-3'-azidothymidine-5'-(p-bromophenyl) methoxyalaninyl phosphate] (Figure 15B) have been identified to exhibit potent anti-HIV and spermicidal activity [91,92]. They are dual-functional microbicides lacking detergent-type membrane toxicity, which would have advantages over the currently available vaginal microbicides. Unlike, N-9, the spermicidal activity of WHI-05 and WHI-07 in mice and rabbits indicated these are non-cytotoxic and lack of inflammation-inducing properties [93,94].

D'Cruz et al. also demonstrated that an intravaginal application of 2% WHI-07 via a gel microemulsion in rabbit model resulted in marked contraceptive activity [95].

Vanadocenes: Spermicidal organometallic complexes of vanadium (IV) with bis(cyclopentadienyl) rings, called vanadocenes are a new class of experimental contraceptive agents. These are reported to have rapid, potent and selective sperm immobilizing activity (SIA) at nanomolar to micromolar concentrations. The SIA of representative vanadocenes was 400-fold more potent than that of N-9. Vanadocene dihalides immobilized human sperm in semen within 15 sec without affecting the sperm membrane integrity or viability of normal human vaginal or cervical epithelial cells. These features of vanadocenes fundamentally differ from those of currently used membrane-active detergent-type spermicides that are cytotoxic to genital tract epithelial cells at spermicidal concentration. The lack of detergent-type membrane toxicity of spermicidal vanadocenes suggests that they may have particular clinical utility as a new class of contraceptive agents. Spermicidal activity of vanadocenes were shown to be mediated by a unique mechanism involving membrane intercalation that was independent of dynein adenosine triphosphatase activity, protein tyrosine phosphatase activity, and the phosphocreatine/creatine kinase system. Among the 45 vanadocenes that were synthesized and evaluated for human spermicidal activity vanadocene acetylacetonato monotriflate (VDACAC) (Figure 16A) and vanadocene dithiocarbamate (VDDTC) (Figure 16B) were the most stable and potent spermicidal vanadocenes [96-103].

Repeated intravaginal application of gel formulations of VDACAC

Figure 15A: WHI-05 [5-bromo-6-methoxy-5,6-dihydro-3'-azidothymidine-5'-(p-methoxy-phenyl) methoxyalaninyl phosphate].

Figure 15B: WHI-07 [5-bromo-6-methoxy-5,6-dihydro-3'-azidothymidine-5'-(p-bromophenyl) methoxyalaninyl phosphate].

Figure 16: Vanadocene. **(A)** vanadocene acetylacetonato monotriflate (VDACAC). **(B)** vanadocene acetylacetonato monotriflate (VDACAC).
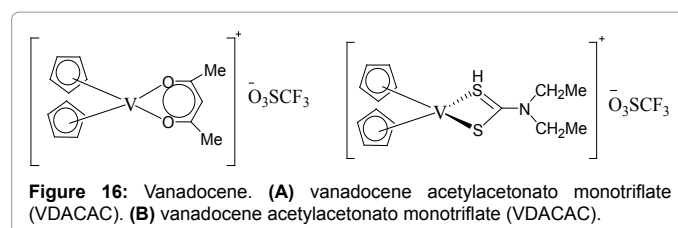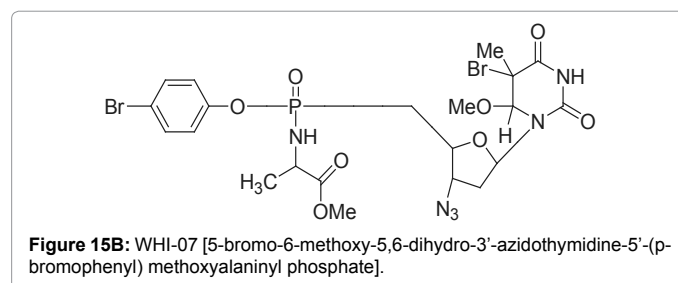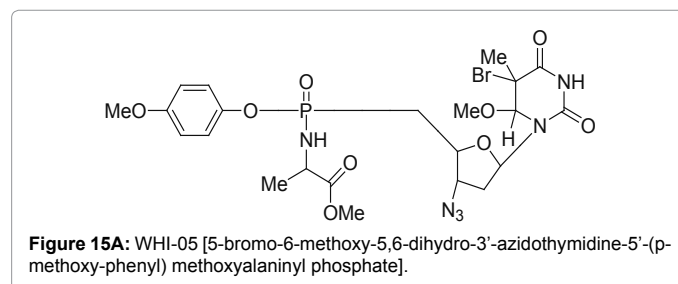
and VDDTC did not induce local inflammation, toxicity or retention of vanadium in the mice and rabbit vaginal irritation model. D'Cruz et al. demonstrated that the intravaginal application of a 0.1% VDDTC in rabbits and pigs results in remarkable contraceptive activity [104-107].

Carbodithionate and carbamodithionate derivatives: These constitute a dually active, nonsurfactant series of molecules (15 compounds) that are capable of killing *Trichomonas vaginalis* (both metronidazole-susceptible and -resistant strains). All of the novel compounds were found to be much safer than N-9 towards Human Cervical Epithelial Cells (HeLa) and vaginal microflora (*lactobacillus*). The most promising candidate, however, was the compound pyrrolidinium pyrrolidine-1-carbodithionate (Figure 17) which killed sperm and trichomonas at concentrations that were about 15 and 450 times lower than its IC_{50} against *lactobacillus*. The study clearly indicates a highly specific action and a much better safety index for the new compound compared to N-9 and warrants further investigation for the development of a safe microbicidal contraceptive [108].

C31G: C31G is spermicidal formulation composed of an equimolar mixture of n-dodecyl-dimethylamine-N-oxide ($C12-NMe_2-O$) and N-(n-dodecyl)-N-dimethyl-glycine (C12-betaine) with microbicidal/virucidal properties. C31G has shown *in vitro* activity against a large number of gram-negative, gram-positive bacterial strains as well as few fungal strains. It is a potent virucidal agent with activity against HIV and herpes simplex virus [109]. A phase I double-blind randomized study of 1.2% C31G with hydroxyl ethyl cellulose (HEC) suggested that physical epithelial changes after seven consecutive days of product use were similar to changes seen with a marketed 2% nonoxynol-9 product (Gynol-II). The subjective symptoms of genital burning or heat, however, were much greater with the C31G HEC product, which limits its usefulness [110].

Disulphide esters of carbothioic acid: DSE-36 and DSE-37 synthesized by Jain et al. killed sperm 25 times more strongly and with a more precisely targeted action than nonoxynol-9 (N-9). The effects of DSE-36 (Figure 18A) and DSE-37 (Figure 18B) on human spermatozoa versus HeLa cells were examined to establish specificity and safety compared with N-9 [111-113].

Indole derivatives: 3,3-Diheteroaromatic oxindole derivatives (Figure 19) prepared by coupling indole-2,3-dione (isatin) with differently substituted indoles and pyrrole in presence of I_2 in i-PrOH were evaluated for in vitro spermicidal potentials and the mode of spermicidal action of the synthesized analogues were evaluated. The
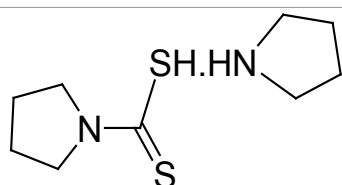

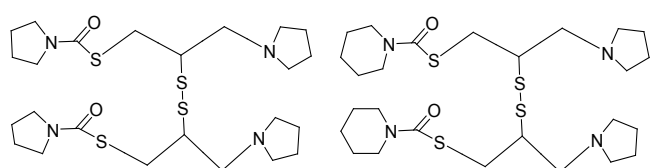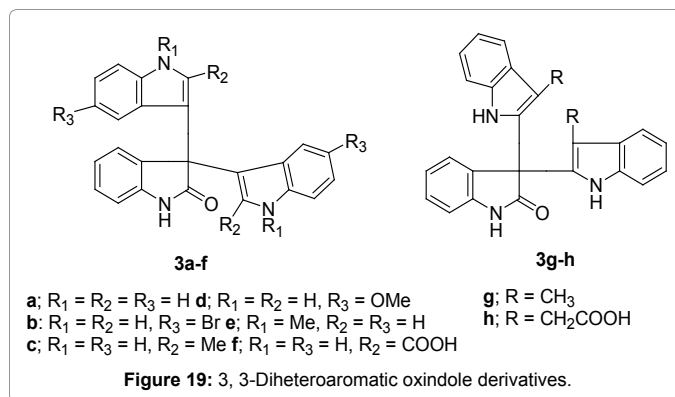**Figure 17:** Pyrrolidinium pyrrolidine-1-carbodithionate.

**Figure 18:** Disulphide esters of carbothioic acid. **(A)** DSE-36. **(B)** DSE-37.

3a-f

a; $R_1 = R_2 = R_3 = H$ **d**; $R_1 = R_2 = H$, $R_3 = OMe$
b; $R_1 = R_2 = H$, $R_3 = Br$ **e**; $R_1 = Me$, $R_2 = R_3 = H$
c; $R_1 = R_3 = H$, $R_2 = Me$ **f**; $R_1 = R_3 = H$, $R_2 = COOH$

3g-h

g; $R = CH_3$
h; $R = CH_2COOH$

Figure 19: 3, 3-Diheteroaromatic oxindole derivatives.

Compound	R
a	H
b	4-Me
c	2-Cl
d	3-Cl
e	4-Cl
f	2-4 Cl_2
g	2Br
h	3Br
i	4-F
j	3-NO_2

1 a-m

2 a-f, h-i, k

3 a-c, f, h, i

Figure 20: Isoxazolecarbaldehydes.

derivative 3, 3-bis (5-methoxy-1H-indol-3-yl) indolin-2-one (**3d**) exhibited the most significant activity [114].

Carbaldehydes: Substituted isoxazolecarbaldehydes (Figure 20) were synthesized by Gupta et al. and established as a new class of compounds exhibiting both spermicidal and acrosin inhibitory activities simultaneously. The authors have indicated that 5- and 3-substituted isoxazole carbaldehydes are 'first generation' multifunctional, spermicidal molecules that hold promise for development as topical contraceptives with useful associated activities that can add considerably to their effectiveness, safety and prophylaxis [115].

Quinoline derivatives: A series of twenty two derivatives of 3-(1-alkyl/aminoalkyl-3-vinyl-piperidin-4-yl)-1-(quinolin-4-yl)-propan-1-one and their 2-methylene derivatives were synthesized from naturally abundant cinchonine. Tartarate salts of these compounds were prepared and evaluated for spermicidal activity. The most active compounds (Figure 21A-E) showing potent spermicidal activity were further evaluated against different strains of *Trichomonas vaginalis* for antimicrobial activity, in HeLa cell lines for cytotoxicity, and against *Lactobacillus jensenii* for eco-safety. The tartarate of 3-(1-pentyl-3-

Figure 21: Derivatives of 3-(1-alkyl/aminoalkyl-3-vinyl-piperidin-4-yl)-1-(quinolin-4-yl)-propan-1-one and their 2-methylene derivatives.

Figure 22: Fused tricyclic oxazepino quinolinium salts.
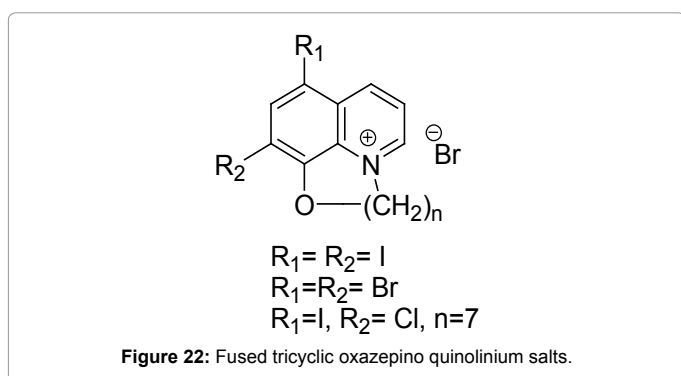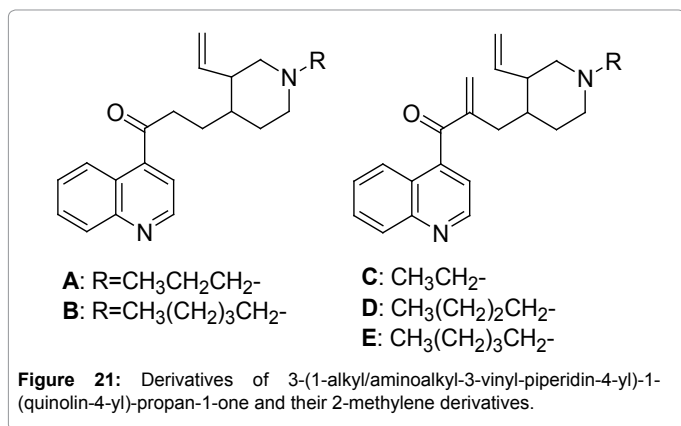
vinyl-piperidin-4-yl)-1-(quinolin-4-yl)-propan-1-one) was found to be more active than N-9 in spermicidal activity [116].

7-Membered oxazaquinolinium salts: Fused tricyclic oxazaquinolinium salts (Figure 22) were synthesized using Amberlite IRA 402(OH) in water and assessed for their sperm-immobilizing efficacy by various in vitro assays. Out of eleven derivatives three showed potent spermicidal effect. Antimicrobial efficacies of the potent derivatives, assessed by disc diffusion as well as by agar and broth dilution methods using human isolates of bacteria and fungi, revealed their moderate antimicrobial potential. The compounds, found to be nontoxic to rabbit erythrocytes by hemolytic assay, may be exploited as microbicidal spermicides [117].
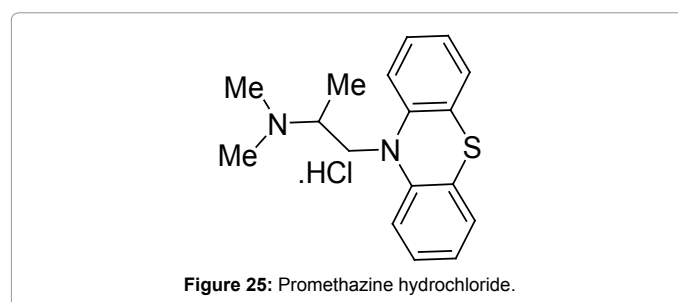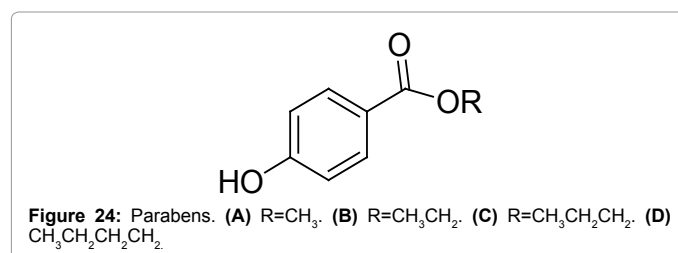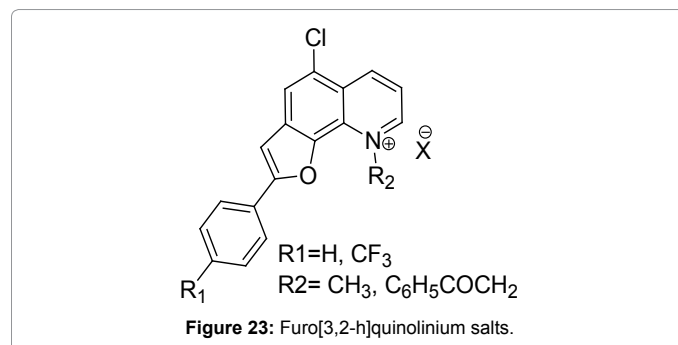
Furo[3,2-h]quinolinium salts: 7-Aryl substituted furo[3,2-h] quinoliniums, which are the hybrids of quaternary pyridinium salts and benzo[*b*]furans, were synthesized in two steps from 5-chloro-8-hydroxy-7-iodo-quinoline and aryl acetylenes through a tandem Sonogashira alkynylation-cyclization pathway followed by quaternisation using methyl iodide/2-bromo acetophenone under MW irradiation using basic alumina. The spermicidal potentials of furo[3,2-*h*]quinoliniums **5d** and **5e** (Figure 23) are at least four to five times higher than the standard spermicide N-9, which signifies their possibility of being used as an alternative to N-9. The compounds destroyed the functional and structural integrity of the sperm plasma membrane with no impact on *Lactobacillus* sp. though these were toxic to common vaginal pathogens. Hence it is believed that these dually active molecules will find application in future formulations for microbicidal contraceptive preparations replacing nonoxynol-9 (N-9) [118].

Parabens: Parabens are a class of chemicals widely used as preservatives by cosmetic and pharmaceutical industries. These are effective preservatives in many types of formulations. These compounds

and their salts are used primarily for their bactericidal and fungicidal properties. Methyl, ethyl, propyl and butyl paraben (Figure 24A-D) have shown potent spermicidal activity at the concentrations of 6, 8, 3, and 1 mg/mL respectively [119].

Gel microemulsions: In 2001, in a systematic effort to develop a dual-function intravaginal spermicide as well as a drug delivery vehicle against sexually transmitted pathogens, D'Cruz et al. formulated novel submicron (30-80 nm) particle, lipophilic and spermicidal gel-microemulsion (GM) formulations GM-144 and GM-4. Microemulsions are thermodynamically stable and isotropically clear dispersions of water, oil, and surfactants with potential as drug-delivery vehicles. GM-144 prepared from seven nontoxic pharmaceutical excipients (propylene glycol, Captex 300, Cremophor EL, Phospholipon 90G, Rhodigel, Pluronic F-68 and sodium benzoate) was found to show rapid sperm-immobilizing activity in human semen in less than 30 sec. GM-4 formulation containing eight pharmaceutical excipients (Captex 300, Cremophor EL, Phospholipon 90G, Propylene glycol, PEG-200, seaspan carrageenan, viscarin carrageenan and sodium benzoate) exhibited potent spermicidal activity in less than 2 min. When tested as vaginal contraceptives in standard rabbit model, GM-144 proved as effective as the commercially available N-9 formulation (Gynol II) and GM-4 was far more effective than Gynol-II. No toxic effect was observed on the vaginal mucosa of rabbits after daily exposure for 10 days [120-123].

Miscellaneous agents: New indolyl phthalazinones and phthalazine derivatives showed marked spermicidal activity, which was ten times that of N-9 [124]. 1, 4-Naphthaquinone was capable of

Figure 23: Furo[3,2-h]quinolinium salts.

Figure 24: Parabens. **(A)** R=CH_3. **(B)** R=CH_3CH_2. **(C)** R=$CH_3CH_2CH_2$. **(D)** $CH_3CH_2CH_2CH_2$.

Figure 25: Promethazine hydrochloride.

generating reactive oxygen species (ROS) and showed spermicidal and antimicrobial activities [125]. Promethazine hydrochloride (Figure 25) showed spermicidal activity at 0.4 mg/mL concentration, and is more effective than propranolol on human sperm [126].
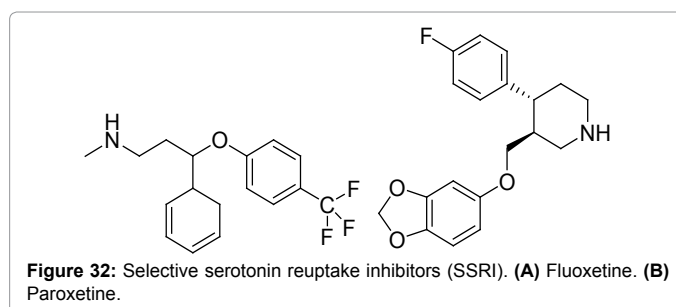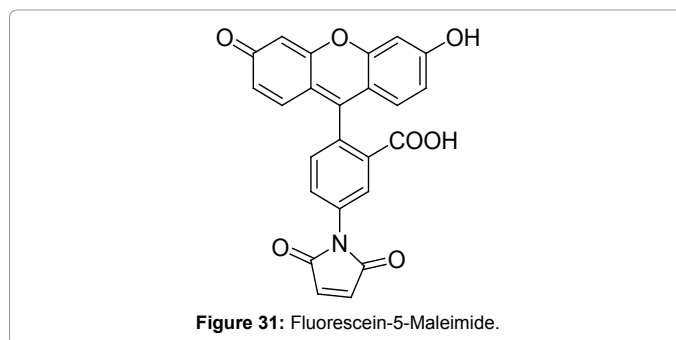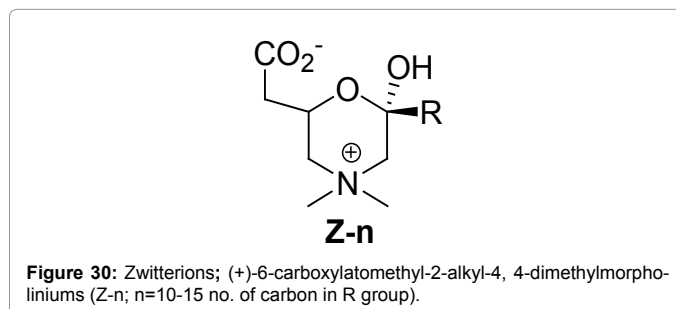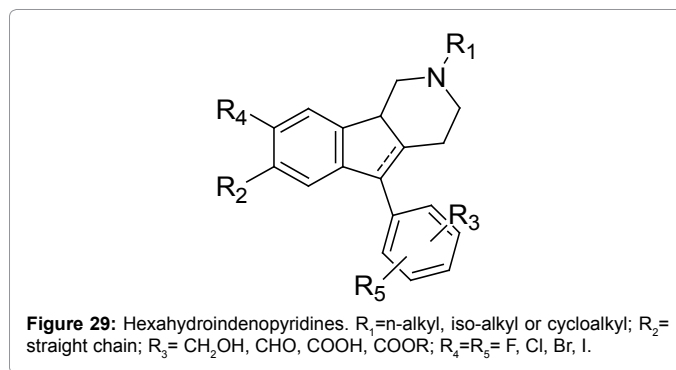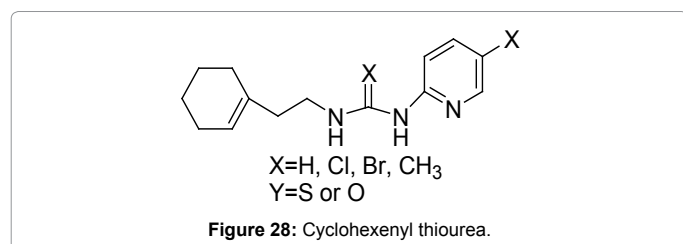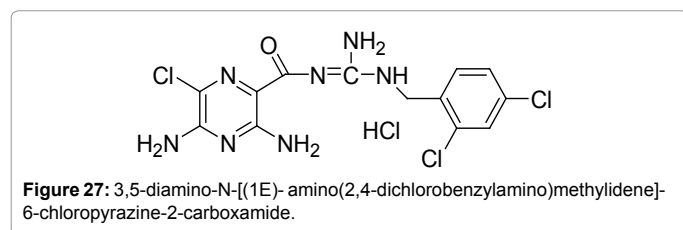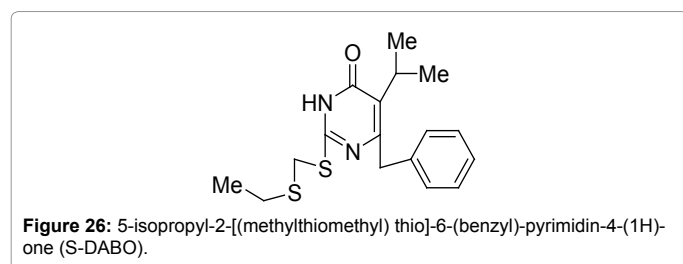
Dihydro alkoxy benzyl oxopyrimidine (DABO) derivatives (Figure 26) exhibited spermicidal activity as well as antiviral activity [127].

A new delivery system (ACIDFORM) was designed with acid-buffering, bioadhesive and viscosity-retaining properties to maintain the acidic vaginal milieu (the low pH inactivates many pathogens and spermatozoa), form a protective layer over the vaginal/cervical epithelium (minimizing contact with pathogenic organisms), and provide long-term vaginal retention [128]. A Phase I clinical study with ACIDFORM provided initial information about its safety and showed the formation of a layer over the vaginal/cervical epithelium [129].

The Na^+-Ca^{2+} exchanger and Ca^{2+}-ATPase pumps reported to be present on the sperm membrane are responsible for maintaining the intracellular Ca^{2+} concentration that is involved in regulation of sperm function. 2',4'-Dichlorobenzamil hydrochloride, (Figure 27) a Na^+-Ca^{2+} exchange inhibitor, showed spermicidal activity [130].

Octyl-D-glucopyranoside (OGP) can immobilize and kill the sperms depending on concentration. Osmond et al. concluded that the phenyl-substituted and cyclohexenyl-substituted thiourea derivatives (Figure 28) can act as potent anti-HIV agents with sperm immobilizing activity and reduced cytotoxicity when compared with N-9, and have the clinical potential to become the active ingredients of a vaginal contraceptive for women who are at high risk for acquiring HIV by heterosexual vaginal transmission [131].

Hexahydro indenopyridines (Figure 29) were synthesized as spermicides and antifungals [132]. Acylcarnitine analogues, (+)-6-carboxylatomethyl-2-alkyl-4, 4-dimethylmorpholiniums

Figure 26: 5-isopropyl-2-[(methylthiomethyl) thio]-6-(benzyl)-pyrimidin-4-(1H)-one (S-DABO).

Figure 27: 3,5-diamino-N-[(1E)- amino(2,4-dichlorobenzylamino)methylidene]-6-chloropyrazine-2-carboxamide.

X=H, Cl, Br, CH$_3$
Y=S or O

Figure 28: Cyclohexenyl thiourea.

Figure 29: Hexahydroindenopyridines. R$_1$=n-alkyl, iso-alkyl or cycloalkyl; R$_2$= straight chain; R$_3$= CH$_2$OH, CHO, COOH, COOR; R$_4$=R$_5$= F, Cl, Br, I.

Figure 30: Zwitterions; (+)-6-carboxylatomethyl-2-alkyl-4, 4-dimethylmorpholiniums (Z-n; n=10-15 no. of carbon in R group).

Figure 31: Fluorescein-5-Maleimide.

Figure 32: Selective serotonin reuptake inhibitors (SSRI). **(A)** Fluoxetine. **(B)** Paroxetine.

(Figure 30) show in vitro activities as spermicides, anti-HIV agents and inhibitors of the growth of *Candida albicans*. Activity improves with increasing chain length [133]. Maleimide derivatives (Figure 31) have been evaluated as spermicidal agents by inhibiting sperm motility [134].

Selective serotonin reuptake inhibitors (SSRI): Fluoxetine (Figure 32A) and paroxetine (Figure 31B) which are antidepressants binding with neuronal 5-HT transporter through sulfhydryl group, have been recently reported as non-detergent spermicides. Other SSRIs such as fluvoxamine maleate, citalopram hydrobromide, and sertraline hydrochloride also show non-detergent spermicidal activities [135].

Major Possible Pathways by Which the Spermicides Work

Change in calcium balance

The fertilization process consists of an acrosomal reaction and sperm-egg fusion, which require extracellular calcium [136-138]. This external triggering mechanism is regulated by a modification of the calcium balance, which is gated by Ca^{2+} channels, and accompanied by a shift in intracellular pH [139]. Ca^{2+} and pH may in turn control the phosphorylation state of functional proteins in the flagellar axoneme activated directly through cAMP and cAMP-dependent kinase [140,141]. Therefore, each step in the calcium-mediated regulatory process could serve as a potential target site for spermicidal action [142]. To interrupt these steps using inhibitors or blocking agents could provide insight into the mechanisms of spermicidal action. For this purpose, we noted the application of chelates like ethylene diamine tetra acetic acid (EDTA), ethylene bis(oxyethylenenitrilo) tetra acetic acid, and gramicidin. They were found to exert spermicidal action against human sperm at the tested concentration range, impeding sperm penetration to the extended cervical space. Kumar et al [143] have studied the activities of sulfonylureas such as Glibenclamide and gliclazide as well as meglitinide analogues such as ripaglinide which produces a dose and time dependent decrease in viability. The reduction in sperm viability was accompanied by an elevation of Ca^{2+} is and was affected by removal of extracellular Ca^{2+}. Studies conducted by Gulati et al. [144] with Miconazole nitrate, clotrimazole and loperamide hydrochloride furnished similar results where enhanced spermicidal activity of miconazole, clotrimazole and loperamide appeared to be due to further mobilization of Ca^{2+} from partially depleted intrasperm Ca^{2+} stores. Synergism of spermicidal activity and intrasperm Ca^{2+} elevation by miconazole or clotrimazole was observed when Ca^{2+} efflux from spermcells was simultaneously inhibited by 20,40-dichlorobenzamil hydrochloride, a $Na^+–Ca^{2+}$ exchange inhibitor. Gupta et al. [145] also studied the Influence of commonly used H_2 receptor antagonists cimetidine, ranitidine and famotidine on sperm viability and intrasperm Ca^{2+} was evaluated in ejaculated human semen samples. All these drugs were found to reduce sperm viability in a dose- and time-dependent manner. This action was accompanied with elevation of intrasperm Ca^{2+}. 2', 4'-dichlorobenzamil hydrochloride (DBZ), a $Na^+–Ca^{2+}$ exchange inhibitor, that is known to elevate intrasperm Ca^{2+}, potentiated the spermicidal action of H_2-receptor antagonists.

Change in pH level:

The spermatozoa are motile between pH 6.7 to 8.5. The normal vaginal flora of healthy women of childbearing age is dominated by *lactobacilli*. *Lactobacillus* produces a number of compounds including lactic acid, hydrogen peroxide, lactacin, and acidolin that maintain a low, acidic pH (3.5–5.0) [146] and thereby protect against the pathogens that cause STIs including HIV [147,148]. When semen enters the vagina, the pH rises to above 6.0 because of the buffering activity of the ejaculate (pH 7.2-8.0) [149]. It is well documented that sperm are sensitive to low pH and acidic solution can immobilize sperm within minutes [150]. This explains the use of lemon juice in ancient times as spermicide, as well as of modern formulations like "ACIDFORM" [128,129].

Sulfhydryl binding agents

Proteins present in the plasma membrane, head and tail region are rich in disulfide linkages. The dynamic conversion of thiols to disulfide linkages forms an essential part of sperm maturation. Sulfhydryl binding agents exert their action by oxidation, alkylation or formation of mercaptides. Hydrogen peroxide, o-iodobenzoate and several hydroquinones are known to destroy the tertiary protein structure by converting the thiol group of cysteine to disulphide linkages. Phenyl mercuric acid is another mercaptide forming agent. Maleimide derivatives have also been evaluated as spermicidal agents by inhibiting sperm motility through sulfhydryl binding. [138].

Surfactants

Surfactants by definition are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. A common lipid bilayer, which contains external, internal and transmembrane proteins, is fundamental feature of the plasma membrane of the sperm. Natural or synthetic surface agents interact with this lipid bilayer, affect the glycoproteins of the cellular membrane, and modify the ionic transport across the membrane, leading to surface changes. These changes, namely vesiculation, vacuolation or dissolution of head region may occur due to stretching, loosening, or breakdown of the membrane and ultimate removal of the acrosome [16]. Saponins are the natural surface active agents widely occurring in many plants and are reported to have profuse spermicidal action as has been discussed earlier. Among the synthetic detergent type spermicides, Nonoxynol-9 is the most common and is present in many marketed formulations. Nandi et al suggested that the double bonds present in nonionic surfactants form allelic radicals that may react with the molecular oxygen present in the membrane and cause LPO [151].

Acrosin inhibition

Acrosin is the major proteinase present in the acrosome of mature spermatozoa. It is a typical serine proteinase with trypsin-like specificity. The active enzyme functions to cause lysis of the zona pellucida, thus facilitating penetration of the sperm through the innermost glycoprotein layers of the ovum. So inhibition of acrosin is one of the pathways by which certain spermicides work. Substituted isoxazole carbaldehydes, for example, act as spermicides by acrosin inhibition [115].

Membrane disruption

Comparable to the effect of typical detergent type spermicides, certain synthetic molecules caused distinct eruptions on the sperm surface in the form of blebbing, vesiculation or exfoliation that occur throughout the body of the spermatozoa or are confined to the acrosomal region. But these molecules being non-detergent in nature are non-irritant to the vaginal epithelium and hence hold promise for being better candidate as vaginal spermicide [28,117,118].

Lipid peroxidation

Mammalian sperm membrane comprises of high amounts of polyunsaturated fatty acids, plasmalogens, and sphingomyelins that render it highly susceptible to damage by reactions with molecular oxygen. ROS produced under normal conditions are efficiently scavenged by antioxidant enzymes such as SOD. However when ROS levels exceed the antioxidant capacity of sperm cells, it becomes detrimental. Many spermicidal and spermiostatic compounds act by generating ROS in sperm cells [152-154].

Microbicidal Spermicides - A Need of the Day

Each year, of the estimated 210 million pregnancies that occur worldwide, 40% are unplanned [155]; on the other hand, more than 90% of all adolescent and adult HIV infections have resulted from heterosexual intercourse [156]. Thus, there is a growing interest in combining contraception with protection against STIs/HIV. A new

generation of dual-function spermicides that offer protection against STIs (including HIV) and pregnancy and whose action is mechanism-based are urgently needed by the society [157,158]. Taking into consideration the current global need, microbicidal property of any spermicide is of prime importance, but its adverse effects on vaginal health should never be underestimated. Any spermicide should not potentially disrupt the vaginal ecology while exerting its intended action. Especially, inactivating friendly microbes like *Lactobacilli* can bring severe detrimental effects to the normal vaginal ecology. So, it is extremely important to identify and evaluate new contraceptive antimicrobial agents that can be used vaginally in effective doses without causing overt vaginal irritation or other toxicity. This need for the new generation dual action spermicides is also augmented by the fact that currently the use of OCPs and IUDs is becoming restricted the world over due to increased awareness of the associated side-effects. The use of vaginal contraceptives as local (topical) agents in a need-based manner (not requiring regular intake/ application) makes them relatively much safer [159]. Male condoms being the most trusted and easy method of contraception suffers the drawback of not being women controlled. Moreover microbicidal spermicide can also be used as an adjunct to condoms or in medicating condoms to increase their efficacy and safety [160].

Conclusion

The above facts and evidence in the foregoing literature highlight that there is a huge vacant space in the field of contraceptive research to devise newer, safer, and more convenient agents to bring down fertility while providing protection against microbial infections. Vaginal spermicides appear to be the best choice from all these considerations. An ideal preparation is required for the users, especially for the women who can use it as per their own need and convenience, and it must meet following four criteria:

- Rapid action and effective in killing all sperm on contact or rendering them incapable of fertilization,

- Systemic nontoxicity, and nonirritating nature to vaginal and penile skin and mucosa,

- No embryotoxic or fetotoxic effects, and

- Absence of long-term toxicities.

The spermicides work by blocking entry of sperm into the uterus with foam or thick cream and creating an environment in which sperm cannot survive. In surveys of spermicide users, women have been found to appreciate spermicidal products because they not only prevent conception, but improve sexual satisfaction and are convenient to use. Male users also appreciated the additional lubrication the products provide. Inadequate lubrication during sexual activity is a major factor causing rupture of condoms; vaginal spermicides that contribute to vaginal lubrication reduce risk of pregnancy and STDs; and thus the spermicides are well accepted by a section of people [161]. However, with use of spermicides according to directions and at a rational interval, vaginal spermicides will be essentially free of adverse effects. People have used them for several decades and the relative paucity of reports is a testimony to a low incidence of adverse reactions. However, there are also some reported problems that are covered in the medical literature [162].

How well spermicides prevent pregnancy is highly variable depending on whether they are used correctly and consistently. Their effectiveness also depends upon whether they are used in addition to another barrier method, such as the diaphragm, cervical cap, or condom. If used perfectly, the failure rate may be as low as 3%. However, in typical users, the failure rate can be as high as 36%. Studies have shown that when spermicides are used alone, pregnancy usually occurs in 21-26% of women during the first year of spermicide use [163]. The number of pregnancies is significantly reduced when spermicides are used with another barrier method, particularly the condom. Future research therefore needs to be directed to develop new spermicides which will be virtually free of undesirable side-effects while being effective and possess microbicidal activity to prevent STDs but be benevolent to friendly bacteria. They need to be used in conjunction with physical barrier methods to ensure higher rate of success and greater acceptance to the population at large.

References

1. United Nations Population Fund (2011) State of the world population 2011, New York.

2. United Nations, Department of Economic and Social Affairs, Population Division (2011) World population prospects: the 2010 revision, Highlights and advance tables.

3. Singh S, Darroch JE, Ashford LS, Vlassoff M (2011) Adding it up: the costs and benefits of investing in family planning and maternal and newborn health. New York: Guttmacher Institute and United Nations Population Fund.

4. Harrison PF, Rosenfield A (1996) Contraceptive Research and Development: Looking to the Future, Committee on Contraceptive Research and Development, Division of Health Sciences Policy, Institute of Medicine. National Academy Press, Washington, D.C.

5. Zaneveld LJ, Waller DP, Anderson RA, Chany C, Rencher WF, et al. (2002) Efficacy and safety of a new vaginal contraceptive antimicrobial formulation containing high molecular weight poly(sodium 4-styrenesulfonate). Biol Reprod 66: 886-894.

6. Zaneveld LJD (1994) Vaginal contraception since 1994: chemical agents and barrier devices. In: Van Look PFA, Perez-Palacios G (Eds), Contraceptive Research and Development. Oxford University Press, UK.

7. Richardson BA, Martin HL Jr, Stevens CE, Hillier SL, Mwatha AK, et al. (1998) Use of nonoxynol-9 and changes in vaginal lactobacilli. J Infect Dis 178: 441-445.

8. Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, et al. (1999) Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis 180: 1863-1868.

9. Rodríguez-Martínez H, Saravia F, Wallgren M, Tienthai P, Johannisson A, et al. (2005) Boar spermatozoa in the oviduct. Theriogenology 63: 514-535.

10. Blandau RJ (1973) Gamate transport in the female mammal. In: Handbook of Physiology.

11. Hunter RFH (1996) How and when and where do spermatozoa gain their fertilizing ability in vivo? Reprod Domest Anim 31: 51–55.

12. Waller DP, Zaneveld LJ, Fong HH (1980) In vitjro spermicidal activity of gossypol. Contraception 22: 183-187.

13. Kim IC, Waller DP, Marcelle GB, Cordell GA, Fong HH, et al. (1984) Comparative in vitro spermicidal effects of (+/-)-gossypol, (+)-gossypol, (-)-gossypol and gossypolone. Contraception 30: 253-259.

14. Ueno H, Sahni MK, Segal SJ, Koide SS (1988) Interaction of gossypol with sperm macromolecules and enzymes. Contraception 37: 333-341.

15. Kimata H, Nakashima T, Kokubun S, Nakayama K, Mitima Y, et al. (1983) Saponins of pericarps of Sapindus mukurossi gaertn and solubilization of monodesmosides by bisdesmosides. Chem Pharm Bull 31: 1998–2005.

16. Dhar JD, Bajpai VK, Setty BS, Kamboj VP (1989) Morphological changes in human spermatozoa as examined under scanning electron microscope after in vitro exposure to saponins isolated from Sapindus mukorossi. Contraception 39: 563-568.

17. Setty BS, Kamboj VP, Garg HS, Khanna NM (1976) Spermicidal potential of saponins isolated from Indian medicinal plants. Contraception 14: 571-578.

18. Dwivedi AK, Chaudhry M, Sarin JPS (1990) Standardization of a new spermicidal agent sapindus saponin and its estimation in its formulation. Indian J Pharm Sci 52: 165–167.

19. Qian YX, Shen PJ, Xu RY, Liu GM, Yang HQ, et al. (1986) Spermicidal effect in vitro by the active principle of garlic. Contraception 34: 295-302.

20. Pakrashi A, Ray H, Pal BC, Mahato SB (1991) Sperm immobilizing effect of triterpene saponins from Acacia auriculiformis. Contraception 43: 475-483.

21. Mahato SB, Pal BC, Nandy AK (1992) Structure elucidation of two acylated triterpenoid bisglycosides from Acacia auriculiformis Cunn. Tetrahedron 48: 6717–6728.

22. Rajasekaran M, Nair AG, Hellstrom WJ, Sikka SC (1993) Spermicidal activity of an antifungal saponin obtained from the tropical herb Mollugo pentaphylla. Contraception 47: 401-412.

23. Madhu DC, Nair GRN (2001) Antiulcer, antibacterial and spermicidal activities of Salannin. Indian Drugs 38: 629–632.

24. Rithaporn T, Monga M, Rajasekaran M (2003) Curcumin: a potential vaginal contraceptive. Contraception 68: 219-223.

25. Kumar S, Naskar S, Hazra A, Sarkar S, Mondal NB, et al. (2009) Evaluation of spermicidal potential of labdane diterpenes from Andrographis paniculata. Int J Biol Chem Sci 3: 628–663.

26. Saha P, Majumdar S, Pal D, Pal BC, Kabir SN (2010) Evaluation of spermicidal activity of MI-saponin A. Reprod Sci 17: 454-464.

27. Das N, Chandran P, Chakraborty S (2011) Potent spermicidal effect of oleanolic acid 3-beta-D-glucuronide, an active principle isolated from the plant Sesbania sesban Merrill. Contraception 83: 167-175.

28. Bharitkar YP, Banerjee M, Kumar S, Paira R, Meda R, et al. (2013) Search for a potent microbicidal spermicide from the isolates of Shorea robusta resin. Contraception 88: 133-140.

29. Chakraborty D, Maity A, Jha T, Mondal NB (2014) Spermicidal and contraceptive potential of desgalactotigonin: a prospective alternative of nonoxynol-9. PLoS One 9: e107164.

30. Kumar S, Biswas S, Mandal D, Roy HN, Chakraborty S, et al. (2007) Chenopodium album seed extract: a potent sperm-immobilizing agent both in vitro and in vivo. Contraception 75: 71-78.

31. Kumar S, Chatterjee R, Dolai S, Adak S, Kabir SN, et al. (2008) Chenopodium album seed extract-induced sperm cell death: exploration of a plausible pathway. Contraception 77: 456-462.

32. Kumar S, Biswas S, Banerjee S, Mondal NB (2011) Evaluation of safety margins of Chenopodium album seed decoction: 14-day subacute toxicity and microbicidal activity studies. Reprod Biol Endocrinol 9: 102.

33. Setty BS, Kamboj VP, Khanna NM (1977) Screening of Indian plants for biological activity: Part VII--Spermicidal activity of Indian plants. Indian J Exp Biol 15: 231-232.

34. Souad K, Ali S, Mounir A, Mounir TM (2007) Spermicidal activity of extract from Cestrum parqui. Contraception 75: 152-156.

35. Reddy PR, Sharma A, Gupta S, Tiwari AK (2002) Contact spermicides as contraceptives: Efficacy and current status. Indian J Pharm Sci 64: 1–9.

36. Riar S, Devakumar C, Ilavazhagan G, Bardhan J, Kain AK, et al. (1990) Volatile fraction of neem oil as a spermicide. Contraception 42: 479-487.

37. Khillare B, Shrivastav TG (2003) Spermicidal activity of Azadirachta indica (neem) leaf extract. Contraception 68: 225-229.

38. Garg S, Taluja V, Upadhyay SN, Talwar GP (1993) Studies on the contraceptive efficacy of Praneem polyherbal cream. Contraception 48: 591-596.

39. Raghuvanshi P, Bagga R, Malhotra D, Gopalan S, Talwar GP (2001) Spermicidal & contraceptive properties of Praneem polyherbal pessary. Indian J Med Res 113: 135-141.

40. Talwar GP, Raghuvanshi P (2005) Process for the preparation of an improved antimicrobial and spermicidal composition. May 27, Application 999/DEL/2003.

41. Bagga R, Raghuvanshi P, Gopalan S, Das SK, Baweja R, et al. (2006) A polyherbal vaginal pessary with spermicidal and antimicrobial action: evaluation of its safety. Trans R Soc Trop Med Hyg 100: 1164-1167.

42. Joshi SN, Katti U, Godbole S, Bharucha K, B KK, et al. (2005) Phase I safety study of Praneem polyherbal vaginal tablet use among HIV-uninfected women in Pune, India. Trans R Soc Trop Med Hyg 99: 769-774.

43. Kamboj VP, Dhawan BN (1982) Research on plants for fertility regulation in India. J Ethnopharmacol 6: 191-226.

44. Banerjee R, Nigam SK (1980) Chemistry of Acacia cocinna and A.caesia Bark. J. of Indian Chem Soc 57: 1043–1049.

45. Sandhyakumary K, Boby RG, Indira M (2002) Impact of feeding ethanolic extracts of Achyranthes aspera Linn. on reproductive functions in male rats. Indian J Exp Biol 40: 1307-1309.

46. Riar SS, Bardhan J, Thomas P, Kain AK, Parshad R (1988) Mechanism of antifertility action of neem oil. Indian J Med Res 88: 339-342.

47. Sinha KC, Riar SS, Bardhan J, Thomas P, Kain AK, et al. (1984) Anti-implantation effect of neem oil. Indian J Med Res 80: 708-710.

48. Serrano H, Garcia-Suarez MD (2001) Sperm aggregation by water extracts from two Bursera species. Arch Androl 46: 15-20.

49. Lohiya NK, Kothari LK, Manivannan B, Mishra PK, Pathak N (2000) Human sperm immobilization effect of Carica papaya seed extracts: an in vitro study. Asian J Androl 2: 103-109.

50. Chaturvedi M, Dixit VP (1997) Antifertility effects of Citrullus colosynthis Schrad. in male albino rats. Indian J of Environ Sci 1: 89–92.

51. Primorac M, Sekulovia D, Antoni S (1985) In vitro determination of the spermicidal activity of plant saponins. Pharmazie 40: 585.

52. Bandara BMR, Jayasinghie L, Karunaratne V (1989) Ecdysterone from Stem of Diploclisia glaucescens. Phytochemistry 28: 1073–1075.

53. Chaturvedi M, Mali PC, Dixit VP (1995) Antifertility effects of the roots of Echinops echinatus Roxb in male rats. J of Environ Pollut 2: 153–157.

54. Chaturvedi M, Mali PC, Dixit VP (1995) Fertility regulation in male rats with the help ofEchinops echinatus Roxb root extract. J Phyto Res 8: 115–118.

55. Gupta S, Sanyal SN, Kanwar U (1989) Antispermatogenic effect of embelin, a plant benzoquinone, on male albino rats in vivo and in vitro. Contraception 39: 307-320.

56. Guerrero C, Campos G, Taboada J (1988) Chemical studies on Eupatorium brevipes and some biological activity of brevipenin. Rev Latinoam Quim 19: 147–149.

57. Pant G, Panwar MS, Rawat MS, Negi DS (1988) Spermicidal glycosides from Hedera nepalensis K. Koch (inflorescence). Pharmazie 43: 294.

58. Ghosal S, Srivastava AK, Srivastava RS, Chattopadhyay S, Maitra M (1981) Justicisaponin-I, a new triterpenoid saponin from Justicia simplex. Planta Med 42: 279-283.

59. He X, Zhou Y, Huang Z (1992) Study on alkaloids of Melodinus fusiformis. Hauxue Xuebao 50: 96–101.

60. Rawat MS, Negi DS, Pant G, Panwar MS (1988) Spermicidal potential and chemical analysis of Ophiopogon intermedius (rhizomes). Pharmazie 43: 143.

61. Jain GK, Pal R, Khanna NM, Wight et al. (1980) Spermicidal saponin from Pittosporum nighirense Apriott. Indian J of Pharm Sci 42: 12–13.

62. Qian SZ, Zhong CQ, Xu Y (1986) Effect of Tripterigium wilfordii Hook. f. on the fertility of rats. Contraception 33: 105-110.

63. Singh KK, Parmar S, Tatke PA (2012) Contraceptive efficacy and safety of HerbOshieldâ„¢ vaginal gel in rats. Contraception 85: 122-127.

64. Zairi A, Tangy F, Bouassida K, Hani K (2009) Dermaseptins and magainins: antimicrobial peptides from frogs' skin-new sources for a promising spermicides microbicides-a mini review. J Biomed Biotechnol 2009: 452567.

65. Edelstein MC, Gretz JE, Bauer TJ, Fulgham DL, Alexander NJ, et al. (1991) Studies on the in vitro spermicidal activity of synthetic magainins. Fertil Steril 55: 647-649.

66. Reddy KV, Shahani SK, Meherji PK (1996) Spermicidal activity of Magainins: in vitro and in vivo studies. Contraception 53: 205-210.

67. Reddy VR, Manjramkar DD (2000) Evaluation of the antifertility effect of magainin-A in rabbits: in vitro and in vivo studies. Fertil Steril 73: 353-358.

68. Clara A, Manjramkar DD, Reddy VK (2004) Preclinical evaluation of magainin-A as a contraceptive antimicrobial agent. Fertil Steril 81: 1357-1365.

69. Wojcik C, Sawicki W, Marianowski P, Benchaib M, Czyba JC, et al. (2000) Cyclodextrin enhances spermicidal effects of magainin-2-amide. Contraception 62: 99-103.

70. Aranha C, Gupta S, Reddy KV (2004) Contraceptive efficacy of antimicrobial peptide Nisin: in vitro and in vivo studies. Contraception 69: 333-338.

71. Shah V, Doncel GF, Seyoum T, Eaton KM, Zalenskaya I, et al. (2005) Sophorolipids, microbial glycolipids with anti-human immunodeficiency virus and sperm-immobilizing activities. Antimicrob Agents Chemother 49: 4093-4100.

72. Gross RA, Shah V, Doncel GF (2005) Spermicidal and virucidal properties of various forms of sophorolipids. Sept 29, WO 2005089522 A2.

73. Azim A, Shah V, Doncel GF, Peterson N, Gao W, et al. (2006) Amino acid conjugated sophorolipids: A new family of biologically active functionalized glycolipids. Bioconjug Chem 17: 1523-1529.

74. Huwe P, Diemer T, Ludwig M, Liu J, Schiefer HG, et al. (1998) Influence of different uropathogenic microorganisms on human sperm motility parameters in an in vitro experiment. Andrologia 30 Suppl 1: 55-59.

75. Yin CP, Liu JH, Li JG, Zhang YC (1990) Influence of Streptococcus viridans on human sperm progressive motility in vitro. Acta Universitatis Medicinae Tong Ji 28: 512–14.

76. Jiang J, Lu DY (1996) Detection of bacteria from semens of infertile males and their seminal parameters. Chin J Androl 10: 196–198.

77. Ohri M, Prabha V (2005) Isolation of spermatozoal agglutinating factor from Staphylococcus aureus isolated from a woman suffering from unexplained infertility. Fertil Steril 84: 1539–1541.

78. Aggarwal J, Prabha V (2006) Contraceptive effect of sperm-agglutinating factor isolated from Staphylococcus aureus in mouse. BJOG 113: 1039-1043.

79. Reddy PR, Sharma A, Gupta S, Tiwary AK (2002) Contact spermicides as contraceptives: Efficacy and current status. Indian J Pharm Sci 64: 1–9.

80. Stafford MK, Ward H, Flanagan A, Rosenstein IJ, Taylor-Robinson D, et al. (1998) Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. J Acquir Immune Defic Syndr Hum Retrovirol 17: 327-331.

81. Patton DL, Kidder GG, Sweeney YC, Rabe LK, Hillier SL (1999) Effects of multiple applications of benzalkonium chloride and nonoxynol 9 on the vaginal epithelium in the pigtailed macaque (Macaca nemestrina). Am J Obstet Gynecol 180: 1080-1087.

82. Watts DH, Rabe L, Krohn MA, Aura J, Hillier SL (1999) The effects of three nonoxynol-9 preparations on vaginal flora and epithelium. J Infect Dis 180: 426-437.

83. Hooton TM, Fennell CL, Clark AM, Stamm WE (1991) Nonoxynol-9: differential antibacterial activity and enhancement of bacterial adherence to vaginal epithelial cells. J Infect Dis 164: 1216-1219.

84. Brzezinski A, Stern T, Arbel R, Rahav G, Benita S (2004) Efficacy of a novel pH-buffering tampon in preserving the acidic vaginal pH during menstruation. Int J Gynaecol Obstet 85: 298-300.

85. Chantler E, Fisher H, Solanki S, Elstein M (1992) Quantification of the in vitro activity of some compounds with spermicidal activity. Contraception 46: 527-536.

86. Raymond EG, Chen PL, Luoto J; Spermicide Trial Group (2004) Contraceptive effectiveness and safety of five nonoxynol-9 spermicides: a randomized trial. Obstet Gynecol 103: 430-439.

87. Wilkinson D, Tholandi M, Ramjee G, Rutherford GW (2002) Nonoxynol-9 spermicide for prevention of vaginally acquired HIV and other sexually transmitted infections: systematic review and meta-analysis of randomised controlled trials including more than 5000 women. Lancet Infect Dis 2: 613-617.

88. Odku OA, Touitou E (2002) The rationale behind the need to abolish the use of nonoxynol-9, a macrogol ether surfactant, in unprotected sex. Acta Technologiae etLegis Medicamenti, 13: 85–92.

89. Ahmad N, Ziets GA, Das S (1991) Long lasting contraceptive suppository composition and methods of use. March 12, United States Patent No. 4999342.

90. Ladipo OA, De Castro MP, Filho LC, Coutinho E, Waller DP, et al. (2000) A new vaginal antimicrobial contraceptive formulation: phase I clinical pilot studies. Contraception 62: 91-97.

91. D'Cruz OJ, Uckun FM, Venkatachalam T (2002) AZT derivatives exhibiting spermicidal and anti-viral activity. February 21, United States Patent No. 20020022600.

92. D'Cruz OJ, Uckun FM, Venkatachalam T (2002) AZT derivatives exhibiting spermicidal and anti-viral activity. February 28, United States Patent No. 20020025922.

93. D'Cruz OJ, Waurzyniak B, Yiv SH, Uckun FM (2000) Evaluation of subchronic (13 weeks) and reproductive toxicity potential of intravaginal gel-microemulsion formulation of a dual-function phenyl phosphate derivative of bromo-methoxy zidovudine (compound WHI-05) in B6C3F1 mice. Contraception 61: 69–76.

94. D'Cruz OJ, Venkatachalam TK, Uckun FM (2001) Thymidine kinase-independent intracellular delivery of bioactive nucleotides by aryl phosphate derivatives of bromo-methoxy zidovudine (compounds WHI-05 and WHI-07) in normal human female genital tract epithelial cells and sperm. Biol Reprod 64: 51-59.

95. D'Cruz OJ, Uckun FM (2003) Contraceptive activity of a spermicidal aryl phosphate derivative of bromo-methoxy-zidovudine (compound WHI-07) in rabbits. Fertil Steril 79: 864-872.

96. D'Cruz OJ, Ghosh P, Uckun FM (1998) Spermicidal activity of metallocene complexes containing vanadium(IV) in humans. Biol Reprod 58: 1515-1526.

97. Ghosh P, Ghosh S, D'Cruz OJ, Uckun FM. (1998) Structuraland biological characterization of a novel spermicidal vanadium (IV) complex: bis(pi-cyclopentadienyl)- N,N-diethyl dithiocarbamato vanadium (IV) tetrafluoro borate, [VCp2(DeDtc)](BF4). J Inorg Biochem 72: 89–98.

98. D'Cruz OJ, Ghosh P, Uckun FM (1998) Spermicidal activity of chelated complexes of bis(cyclopentadienyl)vanadium(IV). Mol Hum Reprod 4: 683-693.

99. D'Cruz OJ, Vassilev A, Uckun FM (2000) Studies in humans on the mechanism of potent spermicidal and apoptosis-inducing activities of vanadocene complexes. Biol Reprod 62: 939-949.

100. D'Cruz OJ, Uckun FM (2005) Vaginal contraceptive activity of a chelated vanadocene. Contraception 72: 146-156.

101. D'Cruz O, Ghosh P, Uckun F (2002) Vanadium (IV) metallocene complexes having spermicidal activity, July 25, United States Patent No. 20020099087.

102. D'Cruz O, Ghosh P, Uckun F (2003) Vanadium (IV) metallocene complexes having spermicidal activity, January 23, United States Patent No. 20030018068.

103. D'Cruz O, Ghosh P, Uckun F (2005) Vanadium (IV) metallocene complexes having spermicidal activity. September 1, United States Patent No. 20050192266.

104. D'Cruz OJ, Uckun FM (2001) Intravaginal toxicity studies of a gel-microemulsion formulation of spermicidal vanadocenes in rabbits. Toxicol Appl Pharmacol 170: 104-112.

105. D'Cruz OJ, Waurzyniak B, Uckun FM (2001) Subchronic (13-week) toxicity studies of intravaginal administration of spermicidal vanadocene dithiocarbamate in mice. Contraception 64: 177-185.

106. D'Cruz OJ, Uckun FM (2001) Lack of subchronic and reproductive toxicity of intravaginal gel formulations of spermicidal vanadocenes in a 13-week study in B6C3F1 and CD-1 mice. Fertil Steril 76, Supp 1, S17.

107. D'Cruz OJ, Waurzyniak B, Uckun FM (2002) Subchronic (13-week) toxicity studies of intravaginal administration of spermicidal vanadocene acetylacetonato monotriflate in mice. Toxicology 170: 31-43.

108. Jain A, Lal N, Kumar L, Verma V, Kumar R, et al. (2011) Novel trichomonacidal spermicides. Antimicrob Agents Chemother 55: 4343-4351.

109. Thompson KA, Malamud D, Storey BT (1996) Assessment of the anti-microbial agent C31G as a spermicide: comparison with nonoxynol-9. Contraception 53: 313-318.

110. Ballagh SA, Baker JM, Henry DM, Archer DF (2002) Safety of single daily use for one week of C31G HEC gel in women. Contraception 66: 369-375.

111. Jain RK, Maikhuri JP, Kiran Kumar ST, Sharma VL, Dwivedi AK, et al. (2007) Novel disulfide esters of carbothioic acid as potent, non-detergent spermicides with low toxicity to Lactobacillus and HeLa cells in vitro. Hum Reprod 22: 708-716.

112. Jain RK, Jain A, Maikhuri JP, Sharma VL, Dwivedi AK, et al. (2009) In vitro testing of rationally designed spermicides for selectively targeting human sperm in vagina to ensure safe contraception. Hum Reprod 24: 590-601.

113. Jain RK, Jain A, Kumar R, Verma V, Maikhuri JP, et al. (2010) Functional attenuation of human sperm by novel, non-surfactant spermicides: precise targeting of membrane physiology without affecting structure. Hum Reprod 25: 1165-1176.

114. Paira P, Hazra A, Kumar S, Paira R, Sahu KB, et al. (2009) Efficient synthesis of 3,3-diheteroaromatic oxindole analogues and their in vitro evaluation for spermicidal potential. Bioorg Med Chem Lett 19: 4786-4789.

115. Gupta G, Jain RK, Maikhuri JP, Shukla PK, Kumar M, et al. (2005) Discovery of substituted isoxazolecarbaldehydes as potent spermicides, acrosin inhibitors and mild anti-fungal agents. Hum Reprod 20: 2301-2308.

116. Pandey RR, Srivastava A, Malasoni R, Naqvi A, Jain A, et al. (2012) Synthesis of 3-(1-alkyl/aminoalkyl-3-vinyl-piperidin-4-yl)-1-(quinolin-4-yl)-propan-1-ones and their 2-methylene derivatives as potential spermicidal and microbicidal agents. Bioorg Med Chem Lett 22: 5735-5738.

117. Banerjee M, Kumar S, Ghosh S, Paira R, Mondal S, et al. (2014) Amberlite IRA 402(OH)-mediated synthesis and evaluation of fused tricyclic quinolinium salts as potent non-detergent type microbicidal spermicides. Med Chem Res 23: 1488–1500.

118. Banerjee M, Kumar S, Paira R, Ghosh S, Karmakar S, et al. (2014) Synthesis and Characterization of Furo[3,2-h]Quinoliniums as Potent Non-Detergent Spermicides. Letts in Drug Design & Dis 11: 104–113.

119. Song BL, Li HY, Peng DR (1989) In vitro spermicidal activity of parabens against human spermatozoa. Contraception 39: 331-335.

120. D'Cruz OJ, Yiv SH, Uckun FM (2001) GM-144, a novel lipophilic vaginal contraceptive gel-microemulsion. AAPS PharmSciTech 2: E5.

121. D'Cruz OJ, Yiv SH, Waurzyniak B, Uckun FM (2001) Contraceptive efficacy and safety studies of a novel microemulsion-based lipophilic vaginal spermicide. Fertil Steril 75: 115-124.

122. D'Cruz OJ, Uckun FM (2001) Gel-microemulsions as vaginal spermicides and intravaginal drug delivery vehicles. Contraception 64: 113-123.

123. D'Cruz OJ, Waurzyniak B, Yiv SH, Uckun FM (2000) Evaluation of subchronic (13 weeks) and reproductive toxicity potential of intravaginal gel-microemulsion formulation of a dual-function phenyl phosphate derivative of bromo-methoxy zidovudine (compound WHI-05) in B(6)C(3)F(1) mice. Contraception 61: 69-76.

124. Kidwai M, Negi N, Kohli S (1997) New indolyl phthalazinones and phthalazine derivatives as spermicidal agents. Acta Pharm (Zagreb) 47: 53–57.

125. Aitken RJ (1998) Spermicide CA 2283485 A1.

126. Jiang E, Wu Y, Luan SM, Baohua S, Weihuai B, et al. (1999) Experimental study on the spermicidal effect of promethazine hydrochloride in vitro. Acta Academia Medicinae Shandong 37: 79–81.

127. D'Cruz OJ, Uckun FM (1999) Novel derivatives of phenethyl-5-bromopyridylthiourea and dihydroalkoxybenzyloxopyrimidine are dual-function spermicides with potent anti-human immunodeficiency virus activity. Biol Reprod 60: 1419-1428.

128. Garg S, Anderson RA, Chany CJ 2nd, Waller DP, Diao XH, et al. (2001) Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM). Contraception 64: 67-75.

129. Amaral E, Faúndes A, Zaneveld L, Waller D, Garg S (1999) Study of the vaginal tolerance to Acidform, an acid-buffering, bioadhesive gel. Contraception 60: 361-366.

130. Patni AK, Gupta S, Sharma A, Tiwary AK, Garg SK (2001) Role of intracellular calcium in the spermicidal action of 2',4'-dichlorobenzamil, a novel contact spermicide. J Pharm Pharmacol 53: 1387-1392.

131. D'Cruz OJ, Venkatachalam TK, Uckun FM (2000) Novel thiourea compounds as dual-function microbicides. Biol Reprod 63: 196-205.

132. Cook CE, Sloan CD, Thomas BF, Navarro HA (2009) Spermicidal and/or antifungal composition and methods of using the same. US 7544696 B2.

133. Savle PS, Doncel GF, Bryant SD, Hubieki MP, Robinette RG, et al. (1999) Acylcarnitine analogues as topical, microbicidal spermicides. Bioorg Med Chem Lett 9: 2545-2548.

134. Anderson L, Potter WD, Solanki S (2003) Spermicides. WO 2003035059 A2.

135. Kumar VS, Sharma VL, Tiwari P, Singh D, Maikhuri JP, et al. (2006) The spermicidal and antitrichomonas activities of SSRI antidepressants. Bioorg Med Chem Lett 16: 2509-2512.

136. McGrady AV, Nelson L (1974) Effect of calcium on bull spermatozoan membrane potential. Exp Cell Res 87: 398-400.

137. Yanagimachi R (1978) Calcium requirement for sperm-egg fusion in mammals. Biol Reprod 19: 949-958.

138. Sano K, Kanatani H (1980) External calcium ions are requisite for fertilization of sea urchin eggs by spermatozoa with reacted acrosomes. Dev Biol 78: 242-246.

139. Yanagimachi R (1988) Sperm-egg fusion. Curr. Top. Membr. Transp 32: 3-43.

140. Carr DW, Acott TS (1989) Intracellular pH regulates bovine sperm motility and protein phosphorylation. Biol Reprod 41: 907-920.

141. White DR, Aitken RJ (1989) Relationship between calcium, cyclic AMP, ATP, and intracellular pH and the capacity of hamster spermatozoa to express hyperactivated motility. Gamete Res 22: 163-177.

142. Gupta A, Gupta P, Jain S , Moudgil P Tiwari A.K (2005) Modulation of Intrasperm Ca2+: a possible maneuver for spermicidal activity, Drug Development Research 65: 1-16.

143. Kumar N, Jain S, Gupta A, Tiwary AK (2008) Spermicidal activity of sulfonylureas and meglitinide analogues: role of intrasperm Ca2+ elevation. J Pharm Pharmacol 60: 323-330.

144. Gulati A, Tiwary AK, Jain S, Moudgil P, Gupta A (2006) Intrasperm Ca2+ modulation and human ejaculated sperm viability: influence of miconazole, clotrimazole and loperamide. J Pharm Pharmacol 58: 1145-1151.

145. Gupta A, Gupta S, Tiwary AK (2003) Spermicidal efficacy of H2-receptor antagonists and potentiation with 2', 4'-dichlorobenzamil hydrochloride: role of intrasperm Ca2+. Contraception 68: 61-64.

146. Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, et al. (1996) Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. J Infect Dis 174: 1058-1063.

147. Kempf C, Jentsch P, Barre-Sinoussi FB, Poirier B, Morgenthaler JJ, et al. (1991) Inactivation of human immunodeficiency virus (HIV) by low pH and pepsin. J Acquir Immune Defic Syndr 4: 828-830.

148. Klebanoff SJ, Coombs RW (1991) Viricidal effect of Lactobacillus acidophilus on human immunodeficiency virus type 1: possible role in heterosexual transmission. J Exp Med 174: 289-292.

149. Alexander NJ, Baker E, Kaptein M, Karck U, Miller L, et al. (2004) Why consider vaginal drug administration? Fertil Steril 82: 1-12.

150. Olmsted SS, Dubin NH, Cone RA, Moench TR (2000) The rate at which human sperm are immobilized and killed by mild acidity. Fertil Steril 73: 687-693.

151. Nandi B, Roy S, Bhattacharya S, Sinha Babu SP (2004) Free radicals mediated membrane damage by the saponins acaciaside A and acaciaside B. Phytother Res 18: 191-194.

152. de Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C (1997) Reactive oxygen species and sperm physiology. Rev Reprod 2: 48-54.

153. Aitken RJ, Clarkson JS (1987) Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil 81: 459-469.

154. Halliwell B, Gutteridge JM (1990) Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 186: 1-85.

155. Dailard C (1999) Abortion in context: United States and worldwide. Issues Brief (Alan Guttmacher Inst) : 1-6.

156. Forrest JD (1993) Timing of reproductive life stages. Obstet Gynecol 82: 105-111.

157. Trager RS (2003) Microbicides. Raising new barriers against HIV infection. Science 299: 39.

158. Rosenstein IJ, Stafford MK, Kitchen VS, Ward H, Weber JN, et al. (1998) Effect on normal vaginal flora of three intravaginal microbicidal agents potentially active against human immunodeficiency virus type 1. J Infect Dis 177: 1386-1390.

159. Gupta G (2005) Microbicidal spermicide or spermicidal microbicide? Eur J Contracept Reprod Health Care 10: 212-218.

160. Oshio S, Ozaki S, Ohkawa I, Tajima T, Tomomasa H, et al. (1990) Spermicidal activity of menfegol-coated condom. Andrologia 22: 291-296.

161. Hardy E, de Pádua KS, Jiménez AL, Zaneveld LJ (1998) Women's preferences for vaginal antimicrobial contraceptives. II. Preferred characteristics according to women's age and socioeconomic status. Contraception 58: 239-244.

162. Mayo Clinic News Letter (2007): Drugs and Supplements; Spermicides (Vaginal).

163. Fu H, Darroch JE, Haas T, Ranjit N (1999) Contraceptive failure rates: new estimates from the 1995 National Survey of Family Growth. Fam Plann Perspect 31: 56-63.