Chapter IV

The molecular mechanisms of *Trichomonas* infection and proposed mechanisms for the anti-*Trichomonas* activity of promising agents/combinations

Introduction

Vaginal infection of *Trichomonas vaginalis* may take place prior to puberty, but are generally transient and clear up spontaneously. At puberty, the bacterial flora of the vagina acidifies the pH to ~4.5 (Marquardt et al., 2003). Oestrogen increases vaginal secretion and makes it acidic by promoting the breakdown of glycogen to lactic acid. *Lactobacillus acidophilus*, a normal flora in the vaginal environment, is also known to provide vaginal acidity with pH of 4.0 - 4.5 by secreting high level of lactic acid. Despite the changes taking place in the vagina at sexual maturity, it remains hostile for *T. vaginalis* and other potential pathogenic organisms such as *Mycoplasma homonic* and *Gardinella vaginalis* (Marquardt et al., 2003). There is a continuous turnover of epithelial cells and their physiology probably varies with the stage of menstrual cycle (Marquardt et al., 2003). Under the influence of estrogen and progesterone, the vaginal epithelium becomes cornified, a thick mucus is secreted and the epithelium proliferates and becomes infiltrated with leukocytes. The establishment of *T. vaginalis* in the vagina despite the acidic pH and the presence of Lactobacilli is rather interesting (Petrin et al., 1998). *T. vaginalis* is unable to grow appropriately in presence of sufficient numbers of *L. acidiphilus* (Abraham et al., 1996). However, there is an increase in the number of *T. vaginalis* after the reduction of *L. acidophilus* at the end of menstrual cycle and during menopause. The parasite also seems to have a deleterious effect on *L. acidophilus* (Petrin et al., 1998). *T. vaginalis* has been observed to phagocytose bacteria, which includes *Lactobacillus* (Petrin et al., 1998). Another hypothesis
is that proteinases secreted by *T. vaginalis* may destroy the Lactobacilli (Petrin et al., 1998).

**Cytoadherence** is one of the early steps essential for colonization and persistence of a pathogen during infection (Beachey, 1988). Trichomonads’ attachment to the host cells is a prerequisite for the establishment of infection as the organism must overcome the constant secretions of the vagina (Alderete et al., 1995). In addition, the parasite must survive in an adverse host environment, which is nutrient limiting for optimal growth and multiplication (Lehker and Alderete, 1990; Gorrell, 1985), and which contains specific anti-trichomonal immunoglobulin as well as numerous soluble trichomonad proteinases (Alderete et al., 1991). Also, the site of infection of *T. vaginalis* is under constant hormonal influence during the progression of the menstrual cycle (Lopez, 1950). Cytoadherence by trichomonads is dependent on time, temperature and pH (Arroyo and Alderete, 1989). Adherence, however, does not correlate directly with virulence, since virulent strains isolated from asymptomatic patients exhibited wide differences in their ability to adhere to host cells (Krieger et al., 1990). The cell surface of the trichomonad plays a major role in adhesion, host-parasite interaction and nutrient acquisition through the proteins and glycoproteins displayed on the surface (Petrin et al., 1998). Alderete et al. (1995) demonstrated that at least two classes of molecules are directly implicated in the adhesion of *T. vaginalis* to vaginal epithelial cells (VECs): Adhesins and cysteine proteinases (CP).

A major concern is the association of trichomoniasis with preterm deliveries, low birth weight, increased infant mortality and predisposition to HIV/AIDS (Sorvillo and Kerndt, 1998). However, there has been some reluctance in using metronidazole for trichomoniasis in pregnant women, particularly in the United States, because of weakly mutagenic effects in bacteria and the carcinogenic effects detected in rodents (Coustan, 1999; Saurina and McCormack, 1997). In non-pregnant women, metronidazole is the approved US-FDA drug against trichomoniasis by oral route, but is less effective when used vaginally. Also, its non-spermicidal nature makes it ineffective as a vaginal contraceptive.
microbicide. On the other hand, some anti-trichomonal drugs like emetine, quinine and quinacrine have a rather moderate spermicidal activity. However, the potent spermicide N-9 is also a strong anti-trichomonal agent in vitro (Sugarman and Mummaw, 1988). Intra-vaginal use of N-9 has been proposed in metronidazole resistant cases of trichomoniasis (Bassiouni and Riad, 2005), but N-9 has been shown to increase the incidence of HIV and STD infections in users due to its surfactant nature, and therefore, may not be very suitable for the same.

In this chapter, we have studied the anti-trichomonas activity of Sapindus saponins and its combination with DSE-37 as a safer and more effective alternative to N-9/metronidazole for vaginal prophylaxis against Trichomonas infection during sexual intercourse. An attempt was made in this section to suggest molecular mechanisms of Trichomonas infection and proposed mechanisms for the anti-Trichomonas activity of promising agents/combinations. Effect of T.vaginalis on induction of cytotoxicity towards cervico-vaginal cells was assessed. Cytoadherence of trophozoits with host cells and effect on cytoadherence in presence of promising agents was evaluated. Effect of promising agents on motility, morphology, hydrogenosomal membrane potential and cystein protease activity of T.vaginalis were assessed.

Materials and methodology

Chemicals and Reagents

Dimethyl sulfoxide (DMSO), Metronidazol, trypsin, penicillin, streptomycin, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide (MTT), Z-RR-AMC,TLCK,JC-1 were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum was obtained from Hyclone. All culture media and other reagents were purchased from Sigma-Aldrich, USA.

Effect of compounds on viability of T.vaginalis-infected HeLa

The anti-trichomonal activity of microbicidal compounds and their compatibility with cervical (HeLa) cells was assayed by infecting HeLa with Trichomonas
vaginalsi in vitro. Briefly, HeLa cells seeded at $1 \times 10^4$ per well in 48 well culture plates were incubated for 24 hours and thereafter parasites suspended in DMEM + TYM (2:1 with 5% FBS) were added. The ratio of parasites to HeLa was taken 5:1. Control wells contained only DMEM + TYM medium. Candidate drugs to be tested were serially diluted in DMEM + TYM medium and added to the co-cultures and incubated at 37°C in 5% CO$_2$ atmosphere for 12 and 24 hours. Following completion of treatment, medium was replaced with 20μl/well of MTT in PBS, pH 7.4, and incubated for another 2h. Subsequently, 200μl of DMSO solution was added to each well to dissolve the blue formazan crystals produced by the viable cells and the OD was read at 540 nm in a Bio-Tek Microquant microplate reader (Bio-Tek, Winooski, USA).

**Molecular mechanisms of anti-T. vaginalis activity**

**Effect on cytoadherence of trophozoites to host cells**

Measurement of the individual effects of DSE-37 and Saponins, and their combination (DSE+Saponins) on the cytoadherence of *T. vaginalis* to HeLa cells was studied by the standardized procedure (Arroyo et al 1989) with minor modifications. Trichomonads were labeled overnight with [$^3$H] thymidine in TYM medium and thoroughly washed with normal TYM medium to remove unincorporated [$^3$H] thymidine. The cells were then re-suspended in a 1:2 (vol/vol) ratio of interaction medium (TYM:DMEM) and $1 \times 10^6$ [$^3$H] thymidine-labelled trichomonad trophozoites were allowed to interact with HeLa cells at a ratio of 5:1 for 2h at 37°C in the presence/absence of compounds DSE-37 (15 g/ml), Saponin (15 g/ml) or DSE-37+Saponin (30 g/ml), in vitro. 0.036% $N\alpha$-Tosyl-L-lysine chloromethyl ketone hydrochloride (TLCK), the potent CP inhibitor, was used to compare the inhibition in cytoadherence.). 05% DMSO was used as vehical control. Cells were then thoroughly washed with DMEM-TYM (2:1) interaction medium for removal of non-adhered *Trichomonas* cells, dislodged and mixed with the aqueous radioactivity-counting scintillation fluid (ACS II; Amersham, USA), and the radioactivity due to [$^3$H] thymidine was measured on an LS Analyzer 6500 (Beckman Instruments Inc., USA).
Morphological changes in *T. vaginalis*
Trophozoites of *Trichomonas* were treated with a combination of DSE-37 + Saponins (30µg/ml) for 12 hrs against controls treated with vehicle (0.01% DMSO). Morphological changes were analyzed under a phase contrast microscope (Nikon eclipse 80i).

Effect on hydrogenosomal membrane potential of *T. vaginalis*
The loss of hydrogenosomal membrane potential of trophozoits was quantified by flow cytometry using the lipophilic cationic dye JC-1. *Trichomonas* (10x10⁴ cells/ml) were cultured in TYM medium with 10% FCS in the presence or absence of test compounds (DSE-37+Saponin or N-9, 30 g/ml) and incubated for 6 h at 37°C. 0.05% DMSO was used as vehicle control. After incubation, 5µl/ml JC-1 was added from a stock solution in DMSO (1.0 mg/ml) to the cultures and incubated for an additional 10 min and then cells were centrifuged and washed and resuspended in PBS and analyzed by flow cytometry for JC-1 specific fluorescence. The excitation was at 488nm, and emissions for the green and red/orange fluorescence were at 530nm and 570nm, respectively. Fluorescence was measured using a Flow Cytometer (Model: FACS Calibur, BD Biosciences, USA)

Cysteine Protease activity of trophozoits
Trichomonas parasites were treated for 12 h with DSE-37 (15 g/ml), Saponin (15 g/ml), DSE-37+Saponin (30 g/ml) and TLCK (Nα-Tosyl-L-lysine chloromethyl ketone hydrochloride) (360 µg/ml, positive control). 0.05% DMSO was used as vehicle control. After treatment, parasites were washed with ice-cold phosphate-buffered saline and placed in lysis buffer (10 mM Tris-HCl pH 7.5, 10 mM EDTA, 0.5% Triton X-100) for 30 min on ice. Cell lysates were stored at -20°C after quantification of protein in lysates. Cysteine protease activity was determined using Z-RR-AMC (n-carbobenzoxy-arginyl-arginyl-7-amido-4-methylcoumarin) as substrate, essentially as reported by Thomford *et al.*, 1996. The specific substrate is a peptide sequence carbobenzoxy-L-arginyl-arginine (z-Arg-Arg), linked to 7-amino-4-methyl-coumarin (z-R-R-AMC, Sigma Aldrich).
Briefly, 1 μg of protein was added to 980 μl of 0.5 M Tris-HCl, 0.15 M NaCl, 5 mM DTT (pH 7.6), and 10 μl of Z-RR-AMC (1 mg/ml in DMSO, 15 M final concentration) was added and incubated for 1 hour at 37°C. TLCK, the well known CP inhibitor, was used to compare the inhibition in CP activity. Free AMC release, which indicates cleavage of the substrate by the enzyme action, was monitored through fluorescence measurements at an excitation wavelength of 380 nm and emission wavelength of 450 nm using a fluorescence microplate reader (Model: BioTek FLX800TB, Bio-Tek, USA).

**Data analysis**

All experiments were repeated three times and the results were analyzed by one way analysis of variance (ANOVA) using the GraphPad Prism software (Version 3.0). P values < 0.05 were considered statistically significant.

**Results**

**Effect of compounds on viability of T. vaginalis infected HeLa**

![Graph showing viability of Trichomonas infected HeLa by MTT assay. HeLa cells were co-cultured with T. vaginalis (1:5 ratio) in the presence or absence of test compounds DSE-37 + Saponins (30 g/ml) or N-9 at (30 g/ml) for different time periods (0 to 48h). Trichomonas cause time dependent reduction in viability of HeLa cells, DSE-37+Sap (30 g/ml) provide relief from Trichomonas mediated cell death where as N-9 treatment further reduced viability of HeLa.](image-url)
Fig. 4.2: Inverted phase contrast microscopic comparison of the effectiveness of DSE-37 + Saponin and N-9 at different time interval (12h, 24h & 48h) in removing T.vaginalis infection from infected HeLa cells in vitro. [(I) Note the reduction in HeLa cell number after Trichomonas infection, (II) HeLa cells protected from Trichomonas by the presence of DSE+Saponins and (III) HeLa cells undergo necrosis/apoptosis in presence of N-9. [Magnification 200X. Upper left images 400X].

After infection with Trichomonas vaginalis, the viability of HeLa dropped rapidly and drastically by nearly 50% at 24 hours and 80% at 48 hours. The presence of N-9 did not offer any protection against Trichomonas to HeLa cells but in fact further reduced the viability of the latter by another ~20% in 24 hours and ~5% by 48 hours. On the other hand, the combination drug candidate (DSE-37+Saponins) successfully rescued the HeLa cells from the cyto-toxic effects of Trichomonas and maintained ~80% viability between 24 to 48 hours (Fig 4.1). When the
cultures were observed under a microscope. *HeLa* were found completely covered with *Trichomonas* infection at 24 hours. Treatment of these cultures with N-9 at a concentration of 30μg/ml did not rescue the cells from the number of trophozoites but itself severely affected *HeLa* cell structure/viability. *HeLa* cells treated with N-9 + *T. vaginalis* had higher cytotoxicity over *T. vaginalis* alone because in case of N-9 + *T. vaginalis* treatment there were two potent killers of *HeLa*, *i.e.* N-9 and *T. vaginalis*. Although N-9 also kills *T. vaginalis* but the effect is not rapid enough, and hence a combined toxicity against *HeLa* was evident. On the other hand, DSE+saponins at the same concentration effectively controlled trophozoite numbers without any visible damage to *HeLa* (Fig 4.2), indicating its safety as well as quick and potent detrimental effect on *Trichomonas vaginalis*.

**Molecular mechanisms of anti-** *Trichomonas* **activity**

**Effect on cytoadherence of trophozoites to host cells**

![Graph showing cytoadherence of 3H-labeled Trichomonas to human cervical (HeLa) cells](image)

**Fig. 4.3:** Histogram showing the cytoadherence of 3H-labeled *Trichomonas* to human cervical (HeLa) cells in presence of DSE-37 (15 g/ml), Saponin (15 g/ml) or DSE-37+Saponin (30 g/ml), in vitro. TLCK (0.036%), the potent CP inhibitor, was used to compare the inhibition in cytoadherence. The bars represent the mean counts per minute (cpm) ± the standard error (SE) of three independent assays. Significant differences from the control (0.05% DMSO) are indicated as follows: bP <0.01; and cP <0.001.
When compared with vehicle control group (0.05% DMSO), cytoadherence of Trichomonas to HeLa was inhibited ~50.5% (p<0.001) by Sapindus saponins (15 g/ml), ~31% (p<0.01) by DSE-37 (15 g/ml) and ~93% (p<0.001) by the specific cystein protease inhibitor Tosyl Lysyl Chloromethyl Ketone (TLCK, 360 µg/ml, positive control). However the combination drug (DSE-37 + Saponin; 30 g/ml) at 1:1 ratio exhibited an additive inhibition of ~72% (p<0.001). N-9 was not used in this experiment as a positive control since it adversely affected HeLa viability. The combination of DSE-37 + Saponins very effectively reduced the cytoadherence of Trichomonas to HeLa indicating the potent anti-Trichomonal potential of this new drug combination.

**Morphological changes in Trichomonas**

![Control and Combination](image)

**Fig. 4.4:** Changes in morphology of *T. vaginalis* after treatment for 12 hrs with vehicle control (0.05% DMSO) or new drug combination [DSE-37+Saponin (30µg/ml)]. Green arrow indicates normal morphology with flagella, red arrow indicate reduction in size of trophozoits and disappearance of flagella. [Magnification 200X].

Flagella of *Trichomonas* are essential for motility and attachment of trophozoits with host cells. Parasited treated with vehicle control (0.05% DMSO) show normal shape, size, morphology and specific undulating motility. Surface flagella were present in control group. After treatment of parasites with DSE-
37+Saponin (30 g/ml) trophozoites became immotile, got reduced in size and number and were devoid of flagella (Fig 4.4).

**Effect on hydrogenosomal membrane potential of Trichomonas**

![Fig. 4.5](image_url)

**Fig. 4.5:** Effect of Combination (DSE-37+ Saponin) and N-9 treatment on Hydrogenosomal membrane potential of *T. vaginalis*.

![Fig. 4.6](image_url)

**Fig. 4.6:** Histogram showing flowcytometric assessment of JC-1 labelling in *T. vaginalis* after treatment with (I) vehicle control (0.05% DMSO), (II) Combination [DSE-37+Saponin] (30 g/ml) and (III) N-9(30 μg/ml). TLCK was not used in this experiment since the objective was to assess the status of energy metabolism (indicated by hydrogenosomal membrane potential) of freely dividing Trichomonas in presence of the new drug combination. We used TLCK (protease inhibitor) in experiments involving the cystein protease activity of the parasite (Figs 4.5 and 4.7). Fluorescence measured at the excitation wavelength of 488nm, and emissions for the green and red/orange fluorescence were at 530nm and 570nm, respectively. Values are mean ± SE of three independent experiments. Significance of difference from control is denoted by *P<0.001.*
The hydrogenosomal trans-membrane potential was significantly (p<0.001) depolarized after treatment with combination (DSE+Saponins) while the same remained almost unaltered after treatment with N-9 (Fig 4.5). This indicated apoptosis as the primary mechanism of Trichomonal cell-death by DSE+Sap.

**Cysteine Protease activity of trophozoits**

![Graph showing Cysteine Protease activity of trophozoits](image)

**Fig. 4.7:** Inhibition of Cystein protease activity of *T. vaginalis* after treatment with: Control (0.05% DMSO), DSE-37 (15 g/ml), Saponin (15 g/ml), Combination [DSE-37+Saponin] (30 g/ml) and TLCK (360 µg/ml). Fluorescent measured at excitation wavelength of 380 nm and emission wavelength of 450 nm. The bars represent the mean fluorescence ± the standard error (SE) of three independent assays. Significant differences from the control are indicated as follows: aP <0.05; and bP <0.001.

Cysteine protease is a crucial enzyme of parasite for establishment of pathogenicity. As compared with vehicle control group, Sapindus saponins (15 g/ml) did not reduce cystein protease activity significantly but DSE-37 (15 g/ml) alone and in combination with Saponins inhibited CP activity significantly (p<0.05). The positive control TLCK (360 µg/ml) inhibited CP most significantly (p<0.001).
Discussion

*Trichomonas vaginalis* is a very complex organism, from its biochemistry to the mechanisms of pathogenesis. Areas of pathogenesis that have been pursued include defining soluble factors, elucidating contact-dependent relationship between the vaginal epithelium and *T. vaginalis*, and defining how the organism establishes itself in a normally hostile and changing environment. The host-parasite relationship is very complex, and the broad range of clinical symptoms cannot be attributed to a single pathogenic mechanism. All clinical isolates of *T. vaginalis* appear to be capable of infection and disease production.

Approximately 80% of HeLa cells were found to be viable after infection with *Trichomonas vaginalis* when the microbicidal protection was provided by the combination of DSE+Sap. In absence of this protection, *Trichomonas* reduced the viability of HeLa to ~20% in 48 hours. This clearly indicated the microbicidal potential of DSE+Sap. On the other hand, when N-9 was used as a microbicide in this experiment, instead of protection a combined toxicological effect of N-9 and *Trichomonas* was evident, which was additive and much severe. This experiment clearly highlights why (a) N-9 fails to tender any microbicidal protection and (b) increases the incidence of STDs.

The surface of the trichomonad cell is a mosaic of adhesins, receptors to host extracellular matrix proteins, and carbohydrates, which provide the basis for ligand-receptor binding (Bonilha *et al*., 1995). The cell surface of the trichomonad plays a major role in adhesion, host-parasite interaction, and nutrient acquisition, and the proteins and glycoproteins displayed on the surface have functions in this regard (Alderete and Garza, 1985; Alderete and Pearlman, 1984; Heath 1981, Krieger *et al*., 1985). Attachment to host cells is time, temperature, and pH dependent. *T. vaginalis* appears more inclined to parasitize vaginal epithelial cell lines than other cell types *in vitro*. This is not surprising since epithelial cells are likely to be the principal cell type with which the parasite would interact *in vivo* (Alderete and Garza, 1985). The presence of surface expressed adhesins is not sufficient for cytoadherence (Alderete *et al*., 1995). The adhesins on the parasite surface are protected by proteins, from
the family of cysteine proteinases (CP) which get elaborated during normal growth. Expression of adhesin functionality, therefore, requires unmasking of the adhesins by degrading the protective proteins (Alderete et al., 1995). *T. vaginalis* has many cysteine proteinases (CPs) (Bozner and Demes, 1990; Neale and Alderete, 1990). The cysteine proteinase of *T. vaginalis* is known to participate in a variety of important virulence properties (Alderete et al., 1991) such as cytotoxicity (Alvarez-sanchez et al., 2000), hemolysis (Dailey et al., 1990) and cytoadherence (Arroyo and Alderete, 1989). *T. vaginalis* CPs also have the ability to degrade host immunoglobulins G and A (IgG and IgA), both of which are present in the vagina, and thus help in immune response evasion (Provenzano et al. 1995). This indicates the possibility of multiple roles of the proteinase during infection (Neale and Alderete, 1990). Results of the present study indicate that an equimass combination of DSE-37 and saponins inhibits the adherence of *T. vaginalis* to the host cell surface, a prerequisite for the initiation of infection, possibly via inhibiting CPs. CP activity is essentially required for the adherence of *T. vaginalis* to epithelial cells since pretreatment of trichomonads with TLCK, a CP inhibitor, caused marked decline in their ability to adhere to epithelial cells (Arroyo, 1989). Hence it became apparent that the new drug combination reduces cytoadherance by inhibiting the CP activity of trophozoits. In CP assay, TLCK treatment significantly reduced CP activity, which was also reduced significantly by DSE-37 and its combination with Saponins. Since the proteolytic activity of parasite’s CPs is crucial for adherence, nutrition acquisition and virulence, this appears to be one of the primary mechanisms for the anti-Trichomonal activity of the drug combination.

The vaginal mucosa may be a poor nutritional milieu for microbes. Since the ability to synthesize lipids is lacking in *T. vaginalis*, erythrocytes may be a prime source of fatty acids that are needed by the parasite. In addition to lipids, iron is an important nutrient for *T. vaginalis* and may also be acquired via the lysis of erythrocytes (Lehker et al., 1990). Metabolically active parasites are necessary for lysis of erythrocytes. CP inhibitors greatly reduced erythrocyte lysis, which suggests that CPs may be a lytic factor involved in hemolysis (Dailey et al., 1990). The human pathogen *T. vaginalis* lacks conventional mitochondria and
instead contains divergent mitochondrial-related organelles. These double-membrane bound organelles, called hydrogenosomes, produce molecular hydrogen. Phylogenetic and biochemical analyses of hydrogenosomes indicate their common origin with mitochondria. The hydrogenosome, like the mitochondrion, is 0.5 to 1.0 µm in diameter and is surrounded by a double membrane (Benchimol et al., 1983, 1996, Honigberg et al., 1984). The hydrogenosomes are the site of fermentative oxidation of pyruvate (Muller, 1988, 1992), and they produce ATP by substrate-level phosphorylation, produce hydrogen, process half of the carbohydrates of the cell, and contain homologous enzymes common to those found in both prokaryotes and eukaryotes (Lindmark et al., 1989, Marczak et al., 1983). The hydrogenosomes (like the mitochondria) take part in programmed cell-death (Mariante et al., 2003). Hydrogenosomal potential of *T. vaginalis* was determined by JC-1 labelling in the present study. Results indicate that treatment of trophozoits with equimass combination of DSE-37 and saponins resulted in significant depolarization of hydrogenosomal membrane potential, an effect not seen in case of N-9. Thus the combination of DSE-37 and saponins depolarizes the hydrogenosome, which is analogous to the mitochondria of higher eukaryotes and the site of carbohydrate metabolism, to initiate apoptosis like cell-death of the parasites.

In 1959, a β-hydroxyethyl-2-methyl-5-nitroimidazole, commonly referred to as metronidazole, marketed under the trade name *Flagyl®* was found to be highly effective in the systemic treatment of trichomoniasis (Cosar and Julou. 1959). Other nitroimidazoles, although unavailable in North America, were also approved for clinical use in other parts of the world. These include tinidazole (Sucharit et al., 1979), ornidazole (Fugere et al., 1983), secnidazole (Videau et al 1978), flunidazole (Pereyra et al. 1972), nimorazole (Hayward and Roy, 1976), and carnidazole (Chaudhuri and Drogendijk, 1980). These nitroimidazoles are not themselves cytocidal against *T. vaginalis*, but their metabolic products are (Heine and McGregor, 1993). Metronidazole enters the cell through diffusion and is activated in the hydrogenosomes of *T. vaginalis*. Here, the nitro group of the drug is reduced anaerobically by pyruvate-ferredoxin oxidoreductase. This results in cytotoxic nitro radical-ion intermediates that break the DNA strands.
(Tocher and Edwards, 1994). The response is rapid; cell division and motility cease within 1h and cell death occurs within 8h as seen in cell cultures (Nielsen, 1976).

Metronidazole carries risk of causing birth defects in pregnant women (Rosa et al., 1987) and has a low risk of causing cancer (Beard et al., 1979). It does cross the placental barrier and therefore is not indicated for the treatment of trichomoniasis in women who are in the first trimester of pregnancy (Lossick and Kent, 1991). Resistance of T. vaginalis to metronidazole is on the rise. In 1989, the Centers for Disease Control estimated that 5% of all T. vaginalis patient isolates had some level of resistance to metronidazole (Narcisi and Secor, 1996). Organisms with high levels of resistance are difficult to treat and may require very high, toxic amounts of drug. Hence new antitrichomonal agents are needed to treat resistant organisms. Although there are many other nitroimidazoles, but all the nitroimidazoles have similar modes of antimicrobial activity (Lumsden et al., 1988), and so resistance to metronidazole often includes resistance to the other nitroimidazoles (Narcisi and Secor, 1996). It is therefore wiser to consider other potential drugs for the treatment of trichomoniasis. DSE+Sap could be an ideal choice for vaginal use instead of metronidazole (or similar compounds) because (a) it has contraceptive efficacy which was found to be better than the marketed products (containing N-9), and (b) it is capable of eliminating metronidazole resistant and susceptible Trichomonas vaginalis infections.