

RESEARCH ARTICLE

CONTRACEPTIVE VAGINAL SUPPOSITORY CONTAINING NONOXYNOL-9 AND ZINC ACETATE SALT IN A CLINICAL TRIAL

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ABSTRACT

Nonoxynol-9 (N-9) is the most common active ingredient of spermicides. Spermicides containing N-9 are available in many forms, such as jelly, films, suppositories and foams. The two major problems reported on using N-9 spermicide were high failure and high irritation rates which were the main causes of its withdrawing from markets and as we know local methods of contraception (condoms and vaginal suppositories) have high acceptability especial in short term use. We tried to modify N-9 products in a new formula which is safe, and effective. The new preparation based on the addition of low concentrations of zinc acetate salt ($Zn(OAC)_2$) to N-9 that reduce the irritation of mucous membranes on frequent use and increase the efficacy of N-9. The new preparation made in the form of vaginal foaming suppository. It was tested in-vitro and in-vivo. The in-vitro results show a significant increase in efficacy of the combination ($Zn(OAC)_2$ & N-9) than N-9 alone. Additionally, the in-vivo results indicated a significant decrease in failure and irritation rates in the combination than (N-9 alone) market suppository.

Key words: Contraceptive, Nonoxynol-9, Zinc acetate, irritation and efficacy

INTRODUCTION

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, nonirritating to the vaginal and penile mucosa, not have adverse effects on the developing fetus, free from long term topical and systemic toxicity and should not be systemically absorbed^{1,2}. Spermicides as a contraceptive methods have advantages as that they do not depend on high skilled personnel for their prescription and use, they don't interact systemically or interfere with absorption of other drugs, they are used on demand only not on exact time that may be not remembered also it isn't hormones that may disturb woman body by effecting on ovulation, lactation or others³. Spermicides disadvantages appear as higher rate of failure than hormonal methods, vaginal irritation and vaginal secretions which appear mainly on frequent use⁴. N-9 spermicides have a failure rate of 18% per year on perfect use, and 29% under typical use⁵ and irritation rate was recorded 12% of participants sharing in clinical trial at 2000⁶. on comparing advantages to disadvantages we found spermicide can be better contraceptive method than other hormonal method on treating their problems. N-9 which is the most popular spermicide had low acceptability (16.9%) and it offered to women looking for a short term, user-controlled contraceptive⁷.

Zinc acetate is another approved spermicide which has a spermicidal effectiveness in a 1% concentration it appears this is due to the acetate in zinc acetate which can decrease oxygen utilization by sperm⁸. Zinc acetate came over N-9 as it doesn't cause irritation but it can reduce irritation of the mucosal tissue if found due to zinc ion that is effective in preventing or reducing irritation in a concentration of 0.5% which avoid zinc toxicity⁹. Effective concentrations of Nonoxynol-9, benzalkonium chloride, zinc acetate, cupric chloride, cysteamine, tannic acid and propranolol

ranged at least from 0.15 to 1%¹⁰. N-9 spermicidal activity can withstand to 6 hours¹¹. While, zinc acetate spermicidal activity can withstand for only one hour¹². Addition of $Zn(OAC)_2$ to N-9 was a trial to produce a new product has advantages of both spermicides together.

In this study we suggest that the prepared spermicide vaginal suppository contain N-9 and $Zn(OAC)_2$ in concentration 10:1 respectively possess more advantages over presently marketed formulations which contains N-9 alone for the causes that mentioned before. New preparation contains N-9 plus $Zn(OAC)_2$ salt was tested in-vivo and in-vitro in compare with market suppositories containing N-9 alone.

MATERIALS AND METHOD

1. Materials

1. Nonoxynol-9 was purchased from Sigma Aldrich chemical Co. St Louis, Mo, (USA), 2. Zn acetate dihydrate, Sodium lauryl sulfate, Sodium bicarbonate and Anhydrous Citric acid were purchased from El-Gomhouria Pharmaceutical Chemical Co. (Egypt). 3. Polyethylene glycol base (6000, 400) were purchased from El-Nasr Pharmaceutical Chemical Co. (Egypt) and 4. No Gravidia® suppository was purchased from DKT (Egypt).

Semen samples

Fresh human semen Samples having $>65 \times 10^6$ /ml sperm count with $>70\%$ motility and normal sperm morphology. Samples obtained by masturbation into a sterile vial from healthy, young, and fertile donors. Normal ejaculate volume is between 2 and 6 ml, pH is 7.2-8.0 and color is gray yellow.

2. Equipment

1. Electronic balance, Metler Co, (Switzerland). 2. Stainless

steel suppository molds (2.5 cm in heights and 1.5 cm in thickness) self-made. 3. USP tablet dissolution test apparatus II, Hanson research, California, (USA). 4. UV\VIS double beam Spectrophotometer, spectronic, Genesys, Milton Roy Co., (USA). 5. Light microscope, XSP- 13A, Chene MA, (China).

3. Methods

3.1. Suppository formulation

Suppository formulations were prepared from water soluble bases (PEG 400 and PEG 6000) by melting method¹³. The molten base was poured into a mold of torpedo shape then refrigerated and packaged. Each 2 gm. vaginal suppository contains 100 mg N-9 and 10 mg Zn (OAC)₂.

3.2. Drug release measurement of the prepared suppository

Dissolution was conducted in the USP dissolution apparatus 2 operating at 50 rpm using 500 ml of distilled water at 37°C. 5 ml samples were taken at different time intervals and replaced with 5 ml of fresh dissolution medium maintained at the same temperature. Samples were taken with filter-tipped pipette and analyzed spectrophotometrically at 276 nm for N-9¹³, while zinc acetate was analyzed at 550 nm¹⁴. Results were plotted against time in the representative curve. Release of N-9 from the combination in the prepared suppository was compared with N-9 release lonely as reported by Parrott¹²

and determined at the same time intervals so we can study changes made on addition of zinc acetate.

3.3. In-vitro study

Human males participating in this study were fertile semen donors selected after appropriate screening. Three specimens from each of three males were used in this study. Specimens were collected by masturbation following three days abstinence. Following collection, specimens were incubated at 37°C for 15-30 minutes to allow for liquefaction. Semen volume, sperm aggregation and motility percentage were assessed using a light microscope. Sperm motility and aggregation recorded as percent and best spermicide produce 100% sperm immobility and 100% aggregation. Motility was calculated according to the formula:

$$\text{Motility} = \frac{\text{Motile sperms}}{\text{Motile} + \text{non motile sperms}} \times 100$$

Sperm motility and sperm aggregation were measured before and after addition of spermicide on the semen samples after liquefaction and after one hour from liquefaction for testing different spermicides concentrations on it. Three dilutions were prepared from each suppository (with and without zinc acetate) A, B and C as seen in table 1 from each type of suppository and they tested on semen samples for comparing market (No Gravida®) and prepared suppository in different concentrations.

Table 1: Different dilutions of two spermicides formulas of in-vitro testing

Formula	%	Spermicidal amounts (mg)		
		Market suppository		Prepared suppository
		N-9	N-9	Zn(OAC) ₂
A	2	20	20	2
B	1	10	10	1
C	0.5	5	5	0.5

250 µl from each dilution (solution A, B and C) of N-9 alone suppository (market suppository) were added into 50 µl of semen sample. Then start to vortex it at very low speed for 10 seconds. Drop of the mix is immediately examined under microscope. Result scored positive if 100% of sperm are completely immotile within 20 seconds after dilution¹⁵. Repeat the same procedure for the prepared suppositories contain N-9 and Zn (OAC)₂ and determine the difference in results at different concentrations.

3.4. In-vivo study

The study recruited 78 participants referred for using spermicide suppository formulated of N-9 in PEG base (market or compounded) for conception purpose at family planning unite at General Abo-Korkase hospital and General Minia Hospital during the period from July 2010 to August 2011. Study was made to achieve two purposes from using the new spermicide which were decrease failure and irritation rates. This study is randomized prospective clinical trial. Randomization was computerized and blind.

We choose participants which have special criteria and accept the experiment and study^{17, 18}. Participants should know as much as possible about the clinical trial.

Participants in this study were informed and randomized to receive either N-9 suppository preparation or N-9 plus zinc acetate preparation as computer divides them. All participants would be instructed on the use of the test products¹⁸. Method of application is the most important point in clinical work-up as can change results at all; explain method for each volunteer. Verbal and written informed consent was obtained from all volunteers after giving information about the aim of the study and the procedure involved in it¹⁸.

Participants would be followed through 12 menstrual cycles (approximately 13 months) at least and would have 8 study visits and two studies phone call.

All volunteers had comprehensive evaluation full history of taking other contraceptive methods and after using spermicides (market or prepared). Special notes were made of the age, cycle length, literati, parity, previous method of

contraception and lactation. Notes were taken about pregnancy if occur, vaginal irritation, coital problems, cycle irregularities, secretions and other compliances after use¹⁹.

RESULTS AND DISCUSSIN

Prepared suppository with zinc acetate mainly appeared to have similar physical quality properties to the market suppository as weight variations, melting point, hardness and others.

The dissolution studying of the prepared suppository with zinc acetate has high dissolution rate and high drug release profile as it start release with high concentration that reach

≥95% at first 18 min.

The concentrations of N-9 was measured spectrophotometrically at 276 nm and it was increasing manner until it started to make plateau level after 20 min. that was compared to that made for N-9 by Parrott¹³. Zinc acetate was measured spectrophotometrically at 550 nm and it was increasing manner at first 20 min and reaches to ≥95%.

Table (2) and figure (1) illustrate the high dissolution rates of both N-9 and zinc acetate in the prepared suppository. Release start from first 3 min in increasing manner and the maximum amount released were at 18 min.

Table 2: Amount released of N-9 and Zinc acetate salt from the prepared suppository (at first 18 Min).

Time (min)	Amount Dissolved of Zn(OAC) ₂ (mg)	Amount Dissolved of N-9 (mg)
0	0	0
3	2.5	28.0
6	5.7	38.0
9	6.8	63.0
12	8.2	80.0
15	9.5	90.5
18	9.5	98.7

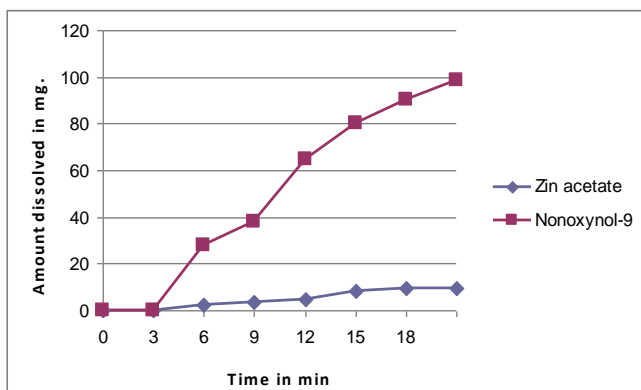


Figure 1: The dissolution rate of N-9 and Zn (OAC)₂ from the prepared suppository

Table (3) and figure (2) show no significant difference in release of N-9 from market suppository as made by Parrote¹³ and that of prepared suppository and so Zn (OAC)₂ don't change N-9 release profile.

Table 3: the amount released of N-9 from market suppository and the prepared suppository at first 18 Min.

Time (min)	Amount released of N-9 (mg) from	
	Market suppository	The prepared suppository
0	0	0
3	28.0	28.0
6	36.0	38.0
9	64.8	63.0
12	81.2	80.0
15	93.5	92.5
18	97.8	98.7

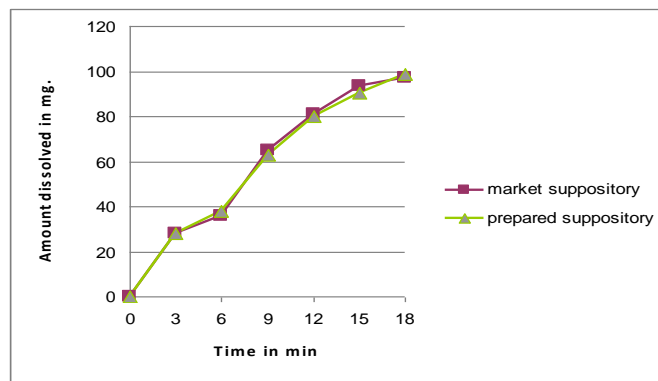


Figure 2: The release of N-9 from market suppository and from prepared suppository

High dissolution rate return to the formula which contains N-9 as it is Non-ionic surfactant²⁰ in non-ionic polymer of PEGs base. Zn (OAC)₂ do not interact with it or impair N-9 activity as the low concentration of Zn (OAC)₂ salt (in compare to N-9 concentration) prevents it from interference with N-9 add to that both active ingredients N-9 and Zn (OAC)₂ are highly stable components and need high temperature degrees to melt or interact.

The in-vitro test made on human semen samples (out human body) for testing changes on sperm motility and sperm aggregation on addition of different concentration of two group of spermicides (with and without Zn (OAC)₂) on it.

Prepared suppository (N-9 plus Zn (OAC)₂) show significant increase in efficacy of the formula in reduction of sperm motility and increasing sperm aggregation than market one in all dilutions especially before first hour passing. As illustrated in table (4).

Table 4: In vitro testing of motility and the aggregation of sperm after mixing with N-9 either alone or with zinc acetate suppository

components concentrations		Sperm motility				aggregation	
		After liquefaction		After one hour			
	Dil.	%	P	%	p	%	p
N-9	-	0		0		100	-
N-9& Zn(OAC) ₂		0		0			
N-9	A	20	0.02*	10	0.1	75	0.5
N-9& Zn(OAC) ₂		0		0			
N-9	B	40	0.16	20	0.13	75	0.5
N-9& Zn(OAC) ₂		20		15		75	
N-9	C	50	0.03*	30	0.16	50	0.3
N-9& Zn(OAC) ₂		20		20		75	

In-vitro test prove the role of zinc acetate addition to N-9 that can increase spermicidal efficacy of the N-9 not diminish it. These results come right with¹⁶ and against⁹.

Different mechanisms of two spermicides (N-9 and Zn(OAC)₂) give the new formula of the combination high strength in reduction of sperm motility. Zinc acetate contains acetate ion which decreases oxygen utilization by sperm that the cause in decrease motility and increase in aggregation.⁸. Nonoxynol-9 vaginal spermicides interact with the lipoproteins of the cell membrane to permanently

disrupt the cell membranes of spermatozoa, resulting in severe damage to the acrosome (head), neck, midpiece, and tail of the sperm and rapid, irreversible loss of function and motility within the vagina and viability¹¹. Different mechanisms give the synergistic effect.

In the clinical studying, When discuss the results related to reduction of irritations we found that after application of drug on group I (market supp.) and group II (prepared supp.) as seen in table (5)

Table 5: Rate of vaginal irritation in both groups of participants of clinical trial

Vaginal Irritation	Group I No. (%)	Group II No. (%)	Total No. (%)	p-value
Yes	8 (22.8)	1 (8.1)	9 (12.5)	0.02*
No	27 (77.2)	36 (97.9)	63 (87.5)	
Total	35 (100)	37 (100)	72 (100)	

The results were observed in the positive side as vaginal irritation decrease with a high significant difference (P=0.02) when zinc acetate salt added to N-9. Irritation in this context may be evidenced by redness or other changes in coloration, inflammation or swelling, hypersensitivity, the occurrence of burning, itching or other painful stimuli. So, zinc ion that is effective in preventing or reducing irritation in a concentration of 0.5% which avoid zinc toxicity⁹.

The most common satisfied mechanism of zinc salt use as

anti-irritant product was that zinc salts may prevent irritation by zinc ions which can bind to negatively-charged regions exposed on the surface of proteins and alter the charge configuration of the protein and prevent subsequent protein-protein interactions between irritants and exposed mucous membranes., thereby preventing its subsequent binding to the underlying tissue and so prevent irritation¹⁶.

Failure which means pregnancy occur show the following results in the table (6).

Table 6: Failure rate in both groups of participants of clinical trial

Failure	Group I No. (%)	Group II No. (%)	Total No. (%)	p-value
Yes	4 (11.4)	0 (0)	4 (5.5)	0.03*
No	31 (88.7)	37 (100)	68 (94.5)	
Total	35 (100)	37 (100)	72 (100)	

The results show increase in the spermicidal efficacy of N-9, Zn acetate combination than N-9 alone so that Zn acetate spermicidal effect can increase the efficacy of N-9 not reduce it. Failure rate of N-9 also decreased significantly (P value equal 0.03). Zinc acetate (and zinc gluconate on adjusting pH to 7.0) was proven as strong spermicide between zinc salts and useful as a vaginal contraceptive⁸. And we assure on Combining two birth control methods, can increase their effectiveness to 95% or more for less effective methods²¹.

CONCLUSION

Nonoxynol-9 is the active ingredient in all of the over-the-counter (OTC) spermicidal products available in the markets and has been used for pregnancy prevention since the 1950s. but it start to be withdrawn from markets after compliance from it's high failure rate and low safety due to high irritation which lead to wounds and lesions that increase rate of sexual transmitted diseases. After screening in family planning clinics we found that local methods of contraception have high acceptability and high fair in the same time so, we tried to introduce a new formula which safe and effective. The new formula contains 0.5% concentration zinc acetate and 5% N-9 concentration which could produce a new spermicidal product with the best quality to the market as two components are approved spermicides at the mentioned concentrations. Different zinc salts as zinc lactate, zinc gluconate, zinc acetate, and other water-soluble organic zinc salts can reduce irritation caused by surfactants (nonoxynol or octoxynol) and other

microbicides in topical genital formulations with different degrees but zinc acetate is the only spermicidal without any changes in its structure. Zinc-containing additives additionally can stabilize and protect cellular membranes, thereby helping protect genital surfaces against damage caused by repeated exposure to agents that attack the lipid membranes that surround mammalian cells. Suitable zinc Salts which have been tested and shown to be non-irritating during sexual intercourse include zinc acetate, zinc propionate, and zinc gluconate. Other zinc salts have also been identified which are soluble in water and have low pK values, which indicates a high rate of zinc ion release²². Our results reached to that addition of Zn (OAC)₂ to N-9 produced an effective spermicide product plus the demulcent effect of zinc ion which appears well at 0.5% concentration that came with^{16,12}.

So we recommended addition of zinc acetate salt to N-9 spermicidal formula to reach the best properties of spermicidal contraceptive.

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