# A new approach in the first-line treatment of bacterial and mycotic vulvovaginitis with topical lipohydroperoxides and glycyrrhetic acid: a comparative study

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**Summary**

*Purpose of investigations:* The aim of this randomized controlled trial was to evaluate efficacy and tolerability of a new association of lipohydroperoxides and glycyrrhetic acid on topical treatment of bacterial and mycotic vulvovaginitis. *Methods:* One hundred consecutive patients with bacterial or mycotic vulvovaginitis were randomly assigned to a study group treated with vaginal lipohydroperoxides and a derivative of glycyrrhetic acid for three days (n = 50), and a control group using vaginal antibacterial metronidazole (500 mg) or antimycotic econazole (150 mg) for six days (n = 50). *Results:* A clinical and microbiological response was achieved in 80.4% and 88.9% in investigational and control group, respectively ($p > 0.05$). Compared to traditional antimicrobial drugs, the effect appears to be faster and safer, even if not significantly. The 6-month recurrence rate was 7.7% and 5.6% in the investigational and control group, respectively. *Conclusion:* Topical medication based on lipohydroperoxides and glycyrrhetic acid showed a clinical and microbiological efficacy in the first-line treatment of bacterial and mycotic vulvovaginitis, comparable to conventional drugs.

*Key words:* Vulvovaginitis; Lipohydroperoxides; Glycyrrhetic acid.

## Introduction

Vulvovaginal infection is a common disease among healthy women and is usually caused by bacteria (Streptococci, Stafilococci, Escherichia coli, Mycoplasma-Ureaplasma, Chlamydia trachomatis, Gardnerella vaginalis), fungi (Candida albicans, glabrata and tropicalis), and protozoa (Trichomonas vaginalis). Current treatment for uncomplicated bacterial and mycotic vulvovaginitis includes a wide range of vaginal antibacterial (metronidazole, tinidazole, clindamycin, etc.) and antimycotic (azoles) preparations, while the oral administration of the same drugs is generally reserved to complicated infections [1].

Ozone is a powerful oxidant principally applied as a disinfectant of drinking and waste water [2-5], but has recently been used in different forms also in several medical indications [6-9]. Ozone damages bacterial nucleic acids with ozonolysis of supercoiled DNA [10], and structural analysis of tRNA has shown that degradation occurs preferentially at guanine residues [11]. Moreover, proteins and lipids are targets in the reactions of ozone with bacterial membranes: ozone fractionates proteins at the tryptophan residues, whereas the reaction with lipids occurs at the carbon-carbon double bonds present in unsaturated fatty acid, producing different toxic products such as hydrogen peroxide, lipohydroperoxides, aldehydes and ozonides [2, 12]. These findings suggest that the ozonized oil activity is due to toxicity rather than to metabolic interruption, as is the case for traditional antimicrobial agents, but with a safety and tolerability profile comparable to essential oils.

The contraindications to conventional antimicrobial drugs and, above all, their overuse with the appearance of "multi-drug resistant" bacterial strains (resistant to two or more antibiotics) [13], has driven research towards the study of alternative drugs such as ozonized oils [14-16]. Sunflower ozonized oil has remarkable bactericidal properties (Mycobacteria, Enterococci, Streptococci, Staphylococci, Escherichia coli, Pseudomonas aeruginosa) as reported by Sechi *et al*. [13] and acts directly on the pathogenic micro-organism without damaging the human epithelium [11]. Application of this system can be more extensive, ranging from the treatment of deep and superficial infections such as those caused by Helicobacter pylori [17] or Staphylococcus aureus and epidermidis [18]. It has been reported that ozonized oil is effective in the treatment of different types of skin diseases caused by Herpes, Mycobacteria, Staphylococci, Streptococci [19-21], and in Candida albicans infections an efficacy of 75% has been reported compared to 81% of treatment with azoles [6]. Different ozonized solutions have also been used successfully against different infections such as otitis, intraocular infections, tinea pedis and vulvovaginitis [5, 7, 8, 22, 23].

The 18-β glycyrrhetic acids (18-βGA), derived from licorice, have been used for their therapeutic properties, including the relief of rheumatic and other pain, and healing gastric ulcers [24, 25]. Moreover, special attention has focused on 18-βGA as an antiviral [26], antimicrobial, against Staphylococcus aureus and Helicobacter pylori [27-29], and antimycotic, against Candida albicans [30, 31].

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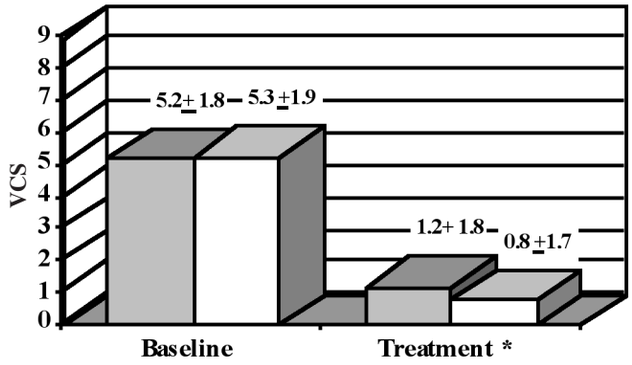
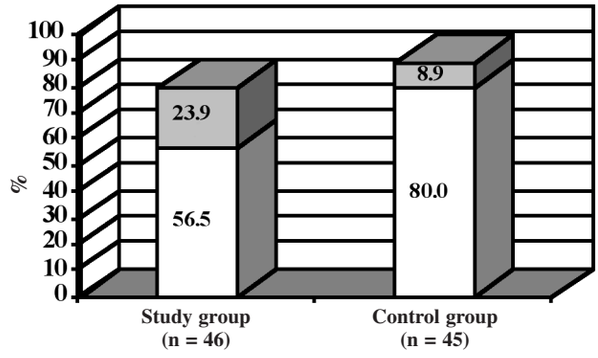



■ Study group (n = 46)** □ Control group (n = 45)
* $p < 0.05$ *vs* Baseline; ** $p > 0.05$ *vs* Control group

*VCS: vulvovaginal clinical score = Burning + Itching + Leucorrhea (not present, 0 points; mild, 1 point; moderate, 2 points; severe, 3 points).*

Figure 1. — Efficacy of treatment in the two study groups (VCS), mean ± S.D.




■ Partial response* □ Complete response*
* $p < 0.05$ vs control group (overall response $p > 0.05$)

*VCS: vulvovaginal clinical score = Burning + Itching + Leucorrhea (not present, 0 points; mild, 1 point; moderate, 2 points; severe, 3 points); Complete response: signs and symptoms not present (VCS 0) and vaginal culture test negative; Partial response: signs and symptoms reduced (VCS ≤ 3) and vaginal culture test negative; Overall response: Complete response + Partial response.*

Figure 2. — Efficacy of treatment in the two study groups (clinical and microbiological response), rate.

The purpose of this randomized controlled trial was to test the natural properties of germicide and soothing effects of lipohydroperoxides (active compounds derived from ozonized oil) and a derivative of 18-βGA, and evaluate efficacy and tolerability of this new association on first-line topical treatment of bacterial and mycotic vulvovaginitis.

## Materials and Methods

One hundred consecutive women with a clinical and microbiological diagnosis of bacterial or mycotic vulvovaginitis were randomly assigned to two study groups: a study group (n = 50) treated with vaginal lipohydroperoxides and a derivative of 18-βGA (Bamic, Difass International srl, Italy), one suppository insertion and two cream applications per day for three days; a control group (n = 50) treated with vaginal antibacterial metronidazole (500 mg) or antimycotic econazole (150 mg), one suppository insertion and two cream applications per day for six days. Ten days from the end of the treatment, both groups of patients underwent a short-term follow-up (gynecological examination and microbiological assessment by vaginal culture test), and at six months, a long-term follow-up was performed (gynecological consult).

Exclusion criteria to enrollment were: topic therapy stopped for less than two weeks; recent contact with soaps, perfumes, powders, deodorants, spermicides, lubrificants; and, hypersensitivity versus compounds.

The primary endpoint of the study was to evaluate the efficacy of the treatment in terms of clinical response (subjective: vulvovaginal burning and itching; objective: leucorrhea) and microbiological evidence (vaginal culture test). We estimated the clinical efficacy through the variations of a vulvovaginal clinical score (VCS) including grade of leucorrhea, itching and burning (not present, 0 points; mild, 1 point; moderate, 2 points; and severe, 3 points). Therefore, we defined the response to treatment as: complete response, signs and symptoms not present (VCS 0) and vaginal culture test negative; partial response, signs and symptoms reduced (VCS ≤ 3) and vaginal culture test negative; no change, signs and symptoms present (VCS > 3) and/or vaginal culture test positive.

The secondary endpoints were the evaluation of velocity of clinical response (time from start of treatment to clinical remission), tolerability profile (incidence of adverse events), and relapses of primary infection (recurrence rate at 6-month follow-up in complete responder patients).

Data are presented as mean value ± standard deviation (SD) and were analyzed using the Student's *t*-test. Statistical significance was set at $p < 0.05$.

## Results

The women enrolled had a mean age of 39.7 ± 15.8 years (range 18-69) in the study group, and 37.5 ± 15.0 years (range 20-67) in the control group, with a postmenopausal status in 28.0% and 24.0% of cases, respectively. In the lipohydroperoxide/18-βGA and conventional drug group, the microbiological diagnosis was bacterial vulvovaginitis in 34.0% and 42.0% of cases, and vulvovaginal candidiasis in 66.0% and 58.0% of cases, respectively.

In the study group, 46 patients completed the treatment plan and underwent the 10-day clinical and microbiological control (drop-out 8.0%), while five patients missed the short-term follow-up in the control group (drop-out 10.0%). Furthermore, in the study group 41 patients completed the study with the 6-month consult (overall drop-out 18.0%), while another six patients missed the long-term follow-up in the control group (overall drop-out 22.0%).

Figure 1 shows the clinical efficacy of the treatment expressed by decrease of VCS compared to baseline values. The improvement resulted significant in both study groups ($p < 0.05$), with a reduction of 76.9% and 84.9% in the study and control group, respectively; these variations did not show statistically significant differ-

ences between lipohydroperoxide/18-βGA and conventional drugs (Figure 1).

Figure 2 shows the efficacy of the treatment expressed by rate of clinical and microbiological response. In the study and control group, respectively, a complete clinical and microbiological response was obtained in 26 and 36 cases ($p < 0.05$), a partial clinical response with negative vaginal culture test in 11 and four cases ($p < 0.05$), and only the few residual patients did not respond to treatment presenting signs and symptoms with a VCS > 3 and/or positive vaginal culture test. In spite of the significant differences by type of response (complete or partial) between the two study groups, no statistically significant differences were seen in overall response rate (complete plus partial): 80.4% versus 88.9% (Figure 2).

In 83.8% of cases of clinical response, burning and itching disappeared or decreased in 24 hours and leucorrhea within two to three days in the lipohydroperoxide/18-βGA group, while the effects were less rapid for conventional drugs (77.5% of prompt clinical response), even if not significantly. No adverse events were observed in the study group compared to 6.7% in the control group ($p < 0.05$). At 6-month follow-up, we found a recurrence rate, calculated only on the 72 completely responding patients, of 7.7% in the study group and 5.6% in the control group.

## Discussion

Bacterial and mycotic vulvovaginitis represent one of the main causes of gynecological consults and are generally treated with topical antibacterial and antimycotic drugs [1]. Recently, there has been great concern about drug resistant strains in these conditions [13], and great interest for alternative antimicrobial agents such as ozonized oil products (e.g. lipohydroperoxides) and 18-βGA for their high germicide power and lenitive properties [14-16, 27-31], and for which no resistance condition has been reported so far.

The main in vitro study on the antimicrobial activity of the ozonized sunflower oil [13] reports an efficacy against several strains of different gram-positive and gram-negative bacterial species (Mycobacteria, Enterococci, Streptococci, Staphylococci, Escherichia coli, Pseudomonas aeruginosa) isolated from different sites (skin, pus, eyes, stools). In particular, Mycobacteria were more susceptible than the other bacteria tested and, interestingly, the ozonized oil showed efficacy against multidrug-resistant strains, such as some Mycobacterium tuberculosis strains resistant to rifampicin and isoniazide, and some Staphylococcus aureus and epidermidis strains resistant to penicillin, gentamicin, tetracycline and erythromycin. Another study [18] confirms ozonized oil activity on drug-resistant bacteria (methicillin-resistant strains of Staphylococcus aureus and epidermidis). Moreover, an additional factor avoiding resistance phenomena is represented by 18-βGA because of the antimicrobial activity shown by licorice flavonoids against methicillin-resistant Staphylococcus aureus [28]. A more recent study verified in vitro (against Staphylococcus aureus, Pseudomonas aeroginosa, Candida albicans, Salmonella typhimurium and Escherichia coli) and in animal models (against Staphylococcus aureus), a significant antimicrobial activity, anti-inflammatory and wound-healing properties for ozonized sunflower oil, compared to other antimicrobial agents commercially available [32].

Ozonized oil has been used in vivo for different medical indications, such as skin diseases, otitis, intraocular infections, and tinea pedis [5-7, 19-22]. In gynecology, a controlled study on 30 patients with vulvovaginitis related to Gardnerella vaginalis, Trichomonas vaginalis and Candida albicans, and treated with topical ozonized sunflower oil, reports a 100% cure obtained between five and seven days [23]; the largest series using the same protocol of 320 patients with mycotic or bacterial vulvovaginitis reports the efficacy and tolerability of ozonized sunflower oil compared to conventional drugs [8].

In this study on efficacy and tolerability of topical lipohydroperoxides and a derivative of 18-βGA against bacterial and mycotic vulvovaginitis, 100 consecutive patients were randomly assigned to the investigational group and a control group using conventional antibacterial and antimycotic drugs. Out of the 91 patients completing at least the short-term follow-up, although using a shorter treatment plan (3 days vs 6 days), a clinical response (complete or partial) with negative vaginal culture test was obtained in a statistically similar percentage of cases (80.4% vs 88.9%) in the study and control group, respectively. These findings are in accord with previous studies [8, 23], nevertheless, in our experience the complete response rate was significantly higher in the control group (80.0%) compared to the study group (56.5%). Compared to traditional antimicrobial drugs, the effect of vaginal lipohydroperoxide/18-βGA was faster and safer, even if not significantly, and out of the 72 completely responding patients, the 6-month recurrence rate was 7.7% and 5.6% for the study and control group, respectively.

## Conclusion

In conclusion, our data suggest that bacteria and fungi are susceptible to lipohydroperoxides and 18-βGA, mostly due to the high germicide properties of the lipohydroperoxides. The vaginal preparation based on these compounds has a direct, and not coadjuvant, therapeutic value in bacterial and mycotic vulvovaginitis, even if the rate of complete response is lower than conventional antimicrobial drugs; in this regard, the use lipohydroperoxides and 18-βGA could be a helpful alternative to conventional therapies in case of resistance conditions, low tolerability profile, allergy or contraindications to traditional drugs (e.g. pregnancy).

The results obtained in this study confirm in a larger population our preliminary data [33], and should lead to the setting up of further clinical trials to compare the efficacy of lipohydroperoxides and 18-βGA with other antimicrobial agents.

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