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RESEARCH ARTICLE

EFFECT OF VAGINAL SUPPOSITORIES COMPOSED OF PEPPERMINT OIL AND SUGAR ON CANDIDIASIS AMONG PREGNANT WOMEN

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ABSTRACT

Peppermint oil and hypertonic sugar solution has antifungal effect against candida species. The aim of this study was to determine the effect of vaginal suppositories composed of peppermint oil and sugar at fixed ratio of 1: 2 respectively on signs and symptoms of Vulvo-vaginal Candidiasis (VVC) among pregnant women. The subjects and methods of this study included a convenience sample of 60 pregnant women attending Ante-natal clinics, Tanta University, 30 were study group and 30 were control group. Three tools were used for data collection: Structured interview sheet, Visual Analogue Scale (VAS) and Clinical diagnosis sheet. Results: The present study revealed that there was significant improvement regarding the symptoms and clinical signs of the present VVC and the total mean score of pain intensity in group I, compared to that in group II. Using peppermint oil and sucrose at a fixed ratio of 1: 2 and at concentration of 5 % and 10 % respectively was found to be effective anti- candidiasis. At minimum inhibitory concentration (MIC), cell growth was completely terminated and clear zones of inhibition were observed on agar plates. This study concluded and recommended that vaginal suppositories composed of peppermint oil and sugar is an effective antifungal agent which can be used for treatment of vulvo-vaginal candidiasis during pregnancy without causing side effects. Further studies are needed to support the present study results regarding the effectiveness of the study suppositories on the other types of vaginal infection

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INTRODUCTION

Vulvovaginal candidiasis (VVC) means fungal infection of the vagina. It is also, known as a monilia or yeast infection. (Das et al., 2008) World wide, VVC caused by candida albicans is considered as the most common cause of vulvovaginitis that accounts for 85–90% of cases complain from VVC. (Sobel 1997; Barousse et al., 2005) Nearly 75% of all adult women have had at least one genital "yeast infection" in their lifetimes, and approximately 40% will have recurring episodes. (<http://www.cdc.gov/fungal/candidiasis/genital> 2012; White and Robertson 2012; Akah et al., 2010; Mylonas and Friese 2007) The increase in antimicrobial drug resistance due to overuse of antibiotics along with toxicity and unwanted side effects of many drugs has caused serious problems (Nucci and Marr 2005; Ghannoum and Rice 1999) Polyenes cause serious host toxicity (Kauffman and Carver 1997) whereas azoles are fungistatic and their prolonged use leads to the development of

drug resistance in *C. albicans* and other candida species (Dupont et al., 1996; Sanglard et al., 2003). This has encouraged search for new and less toxic compounds that can be developed as antifungal.

The growth of *Candida albicans* is usually controlled by the normal acidity of the vagina. There are certain factors, such as pregnancy, use of birth control pills or long term antibiotics, diabetes mellitus, poor hygienic care and decreased body immunity can throw off this normal balance of vaginal acidity and lead to an overgrowth of VVC. (Sendid et al., 2007) Pregnant women have a double increase in the prevalence of VVC compared with non-pregnant women. This is due to increased levels of circulating estrogen and deposition of glycogen in the vagina during pregnancy. (Sobel 2007). Overgrowth of VVC can lead to mild to moderate or even severe symptoms of vaginal infection, such as, vulval and vaginal itching, dyspareunia, dysuria, backache and vaginal discharge which is characterized as odorless, thick and white like cottage cheese in its nature. (Achkar and Fries 2010; Barousse et al., 2004) As well as the above symptoms, VVC is associated with inflammation signs such as, vaginal and vulval

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redness, vagina fissuring and edema. (Barousse *et al.*, 2004) A variety of systematic antifungal drugs are currently available to treat VVC, but there is risks for penetrating the placental barrier and entering fetal cord blood leading to serious effects such as, intrauterine growth retardation and preterm labor, so many of these drugs are contraindicated during pregnancy. (Moudgal and Sobel 2003) On the other hand, antifungal drugs are very expensive, linked with some serious side effects and the resistance of fungi to imidazole derivatives. (Barret 2002) So the use of antifungal drugs should depend on appropriate diagnosis and antifungal sensitivity tests. (Yongabi *et al.*, 2009) Early detection, diagnosis and appropriate treatment may improve the pregnant woman, and neonate clinical condition. (Meizoso *et al.*, 2008)

Using of medicinal plants, which have fewer side effects and inexpensively has been recently taken into concern. (WHO 2002; Ajose 2007; Lee *et al.*, 2007) Peppermint oil is one of the most widely used safe essential oils that have antibacterial, antifungal and antimicrobial properties; also it has a soothing and cooling effect on skin irritations. So peppermint oil may help reducing infections and alleviate signs and symptoms associated with VVC. (Miller 2014) Sugar helps the water to moves from the side with less sugar inside the cells to the side with more sugar out side the cells to dilute the solution in an osmotic process. (Kong 2014) This study was conducted to determine the effect of suppositories composed of peppermint oil and sugar formulated by the researchers of this study as natural and safe components on the signs and symptoms associated with VVC among pregnant women.

Aim of the study

To determine the effect of vaginal suppositories composed of peppermint oil and sugar on signs and symptoms of Vulvo-vaginal Candidiasis (VVC) among pregnant women.

Research Hypothesis

Pregnant women with Vulvo-vaginal Candidiasis who use vaginal suppositories composed of peppermint oil and sugar experience less signs and symptoms than those who don't use it.

Research Questions

What is the effect of vaginal suppositories composed of peppermint oil and sugar on Vulvo-vaginal candidiasis among pregnant women?

Subjects and methods

Study design: Non-randomized control single-blind clinical trial was used in carrying out this study.

Setting: The study was conducted at the pharmaceutical technology laboratory for suppositories preparation, pharmaceutical microbiology lab to determine the microbiological effects of suppository using different concentrations of peppermint oil and sugar on vaginal

candidiasis before giving to the pregnant women at ante-natal clinic of Tanta University Hospital.

Subjects: The study included a convenience sample of 60 pregnant women attending ante-natal clinics. They were equally divided into 2 groups a study (30) and a control (30) group according the following criteria.

- Uncomplicated cases of VVC were involved in this study (have mild to moderate signs and symptoms)
- Free from any medical disease (control).
- Don't use any other pharmacological drugs to relive the signs and symptoms of VVC during intervention period.
- Agree to avoid sexual intercourse during intervention period.

First group: 30 pregnant women used vaginal suppositories composed of Peppermint oil and Sugar.

Second group: 30 pregnant women used placebo vaginal suppositories.

Tools of data collection

The following tools were used for data collection:

Tool I: Structured individual interview sheet:

It included five parts:

Part 1: Demographic data of the studied subjects such as name, age, residence, telephone number, and email address.

Part 2: Physical assessment including height, weight and the body mass index (BMI) (was measured according to the following equation $BMI = \text{weight}/\text{height}^2$)

Part 3: Obstetrical history such as gravidity, gestational age and parity.

Part 4: Past history of previous recurrence of Vulvo-vaginal Candidiasis and methods used to treat it.

Part 5: Symptoms of the present Vulvo-vaginal Candidiasis reported by the woman: Vaginal discharge (amount, color, odor and consistency), vulvo-vaginal itching, dysurea, dyspareunia , backache and lower abdominal pain.

Tool II: Visual analogue scale: To assess pain intensity due to pelvic congestion (dysurea, dyspareunia, backache and abdominal pain). (Breivik *et al.*, 2000) According to pain rating scale, a safe reported device consisting of 10 cm straight line, which represented a continuum of pain intensity and verbal anchors at opposite ends representing no pain. Pain intensity was evaluated by asking the women to point on the line the number that represented the intensity of their pain. The scores corresponding to the pain intensity was as follows: No pain (0), Mild pain (1-3), Moderate pain (4-6), Sever pain (7-9), Unbearable (10). This tool was used before and after the use of formulating and placebo vaginal suppositories.

Tool III: Clinical examination sheet to assess the signs of VVC such as, vaginal discharge (amount, color and consistency), vulvo- vaginal redness and vulvo- vaginal edema

MATERIALS AND METHODS

- 1 Once permission was granted to proceed with the study, the researchers started for research application.
- 2 Laboratory investigation was done in pharmaceutical microbiology lab. the antifungal activity of peppermint oil and sugar determined by the Disc Diffusion Method (Mukhopadhyay *et al.*, 2002) *Candida* cells (105 cells/ ml) were inoculated in molten agar at about 40 °C and poured into a Petri plates. Filter discs were placed on solid agar and different concentrations of test compound (peppermint oil and sugar in fixed ration 1:2) dissolved in 10% (dimethyl sulfoxide) DMSO ranged from 2% to 20% on the disc in 10 µl volume. The average diameter of the inhibition zone recorded in millimeters after 3 days. The experiment was performed in triplicate.
- 3 Formula of vaginal suppositories was prepared at the laboratory of Faculty of Pharmacy, Tanta University through application of the following formula :- polyethylene glycol 6000 (PEG 6000) was melted at about 60 °C separately then mixed with the preheated PEG 400 at about 60°C to which the calculated sugar weight (10%) was added ultrasonication (Ultrasonicator, Sonix IV, Model (SS101H), (USA) for about 5 minutes peppermint oil (5%) was then added to the last mixed base and mixed thoroughly then poured into a suppository mold (5g weight) and cooled at room temperature. Two formulae were prepared: the placebo one and the other was the medicated which contains both peppermint oil (5%) and sugar (10%). Placebo suppositories were composed of PEG 400 (45%) and PEG 6000 (55%) and the medicated suppositories were composed the same base as that of placebo with the incorporation of peppermint oil (5%) and sugar (10%).
- 4 Subjects of this study were interviewed during their attendance in ante-natal clinic, Tanta University. Ethical considerations of the study included approval to collect the data from the previous mentioned setting, as well as getting the subjects' consent to participate in the study after explaining the purpose of the study and reading loudly of the informed consent.
- 5 Before embarking on actual study, a pilot study was carried out on six women attending at ante-natal clinic. Those women were excluded from the study sample.
- 6 The tools were revised and submitted to five experts in obstetric and gynecology nursing from faculty of nursing.
- 7 Opinion of experts on tools of the study was analyzed face validity 95% and validity 97%.
- 8 Confidentiality of information, and right to withdraw from the study at any time if desired.
- 9 The study tools have only code number without using subject's name. All collected data were kept in a locked file cabinet.
- 10 Structured individual interview sheet to collect the following data: demographic data, obstetrical history, past history of previous recurrence of vulvo-vaginal candidiasis and the symptoms of the present Vulvo-vaginal Candidiasis.
- 11 Height and weight of the women were measured.
- 12 Vaginal examination was done by the physician and recorded signs of vaginal infection.
- 13 Vaginal suppositories were given to the women after confirmation of their diagnosis and instruct the women to insert it deeply inside the vagina for three consecutive days at bed time.
- 14 Reassessment for the women was done after three days. If women still complain from the signs and symptoms of VVC after finishing treatment course, another three consecutive days of treatment was prescribed for her.

Table 1. Demographic data and body mass index (BMI) of the studied pregnant women (n=60)

| Variables | Group I (n=30) | | Group II (n=30) | | χ^2 | P |
|-----------------------|----------------|-------------|-----------------|-------------|----------|-------|
| | n | % | n | % | | |
| •Age (years): | | | | | | |
| Range | | 20-40 | | 20-42 | | |
| Mean±SD | | 29.13±5.34 | | 30.07±5.57 | | |
| t-test | | | | | 0.662 | |
| P | | | | | 0.510 | |
| •Education level | | | | | | |
| Illiterate | 2 | 6.7 | 2 | 6.7 | 1.945 | 0.857 |
| Elementary education | 3 | 10.0 | 6 | 20.0 | | |
| Preparatory education | 11 | 36.7 | 7 | 23.3 | | |
| Secondary education | 9 | 30.0 | 10 | 33.3 | | |
| University education | 3 | 10.0 | 3 | 10.0 | | |
| Master degree | 2 | 6.7s | 2 | 6.7 | | |
| •Occupation | | | | | | |
| House wife | 15 | 50.0 | 15 | 50.0 | 0.139 | 0.935 |
| Professional work | 7 | 23.3 | 8 | 26.7 | | |
| Worker | 8 | 26.7 | 7 | 23.3 | | |
| •Marital status | | | | | | |
| Married | 29 | 96.7 | 30 | 100 | 0.000 | 1.000 |
| Divorced | 1 | 3.3 | 0 | 0 | | |
| •Residence | | | | | | |
| Rural | 13 | 43.3 | 10 | 33.3 | 1.629 | 0.736 |
| Urban | 17 | 56.7 | 20 | 66.7 | | |
| •BMI: | | | | | | |
| Range | | 26.90-37.78 | | 26.90-38.06 | | |
| Mean±SD | | 32.82±2.55 | | 32.81±3.64 | | |
| t-test | | | | | 0.010 | |
| P | | | | | 0.992 | |

Group I= Pregnant women used vaginal suppositories composed of peppermint oil and sugar. Group II=Pregnant women used placebo vaginal suppositories.

15 The researchers used tool I (part 5), tool II and tool III for follow up the women to assess the effect of formulating vaginal suppositories on the symptoms, pain intensity and the signs of VVC. This was done two days after finishing the course of treatment.

Statistical analysis

The collected data were organized, tabulated and statistically analyzed using SPSS software (Statistical Package for the Social Sciences, version 13, SPSS Inc. Chicago, IL, USA). For quantitative data, the range, mean and standard deviation were calculated. For qualitative data, comparison between two groups and more was done using Chi-square test (χ^2) and Fisher Exact test (FE). For comparison between means of two groups of parametric data of independent samples, student t-test was used. For comparison between means of two groups of non-parametric data of independent samples, Z value of Mann-whitney test was used.

For comparison between means of two related groups (before and after treatment) of non-parametric data, Z value of Wilcoxon Signed Ranks Test was used. Significance was adopted at $p < 0.05$ for interpretation of results of tests of significance. (Dawson and Trapp 2001; Petrie and Sabin 2005)

RESULTS

Table (1): shows the demographic data and body mass index (BMI) of the studied pregnant women. The table shows that the age ranged from 20-40 in group I and from 20-42 in group II, the mean age was 29.13 ± 5.34 and 30.07 ± 5.57 respectively with no significant differences. There were no significant differences between both groups regarding their education level, occupation, marital status, residence and BMI.

Table (2): shows the obstetric history of the studied pregnant women. It shows that the highest percents of the studied group I and group II groups were multigravida 60% and 70% respectively with no significant differences, and belonged to

Table 2. Obstetric history of the studied pregnant women (n=60)

| Variables | Group I (n=30) | | Group II (n=30) | | χ^2 | P |
|-------------------|----------------|------|-----------------|------|----------|-------|
| | n | % | n | % | | |
| •Gravidity: | | | | | | |
| Primigravida | 12 | 40.0 | 9 | 30.0 | 0.292 | 0.588 |
| Multigravida | 18 | 60.0 | 21 | 70.0 | | |
| •Parity: | | | | | | |
| Nullipara | 12 | 40.0 | 9 | 30.0 | 0.705 | 0.704 |
| 1-3 | 15 | 50.0 | 18 | 60.0 | | |
| 4-6 | 3 | 10.0 | 3 | 10.0 | | |
| >6 | | | | | | |
| •Gestational age: | | | | | | |
| First trimester | 9 | 30.0 | 9 | 30.0 | 0.398 | 0.823 |
| Second trimester | 8 | 26.7 | 10 | 33.3 | | |
| Third trimester | 13 | 43.3 | 11 | 36.7 | | |

Group I= Pregnant women used vaginal suppositories composed of peppermint oil and sugar. Group II=Pregnant women used placebo vaginal suppositories.

Table 3. Previous history of vulvo-vaginal candidiasis of the studied pregnant women (n=60)

| Variables | Group I (n=30) | | Group II (n=30) | | χ^2 | P |
|--|----------------|------|-----------------|-------|----------|--------|
| | n | % | n | % | | |
| •Previous complain of vulvo-vaginal candidiasis: | | | | | | |
| Yes | 18 | 60.0 | 16 | 53.3 | 0.77 | 0.794 |
| No | 12 | 40.0 | 14 | 46.7 | | |
| If yes, time of last complain of vaginal infection: | | | | | | |
| Short time ago | 5 | 27.8 | 7 | 43.7 | 2.62 | 0.623 |
| Long time ago | 4 | 22.2 | 1 | 6.3 | | |
| Recently | 7 | 38.9 | 7 | 43.7 | | |
| Did not apply | 2 | 11.1 | 1 | 6.3 | | |
| Symptoms: | | | | | | |
| Vaginal discharge | 18 | 100 | 15 | 76.7 | 0.271 | 0.604 |
| Vaginal itching | 18 | 100 | 14 | 70.0 | 0.601 | 0.437 |
| Dysurea | 11 | 61.1 | 8 | 50.0 | 0.313 | 0.579 |
| Dyspareunia | 13 | 72.2 | 7 | 46.7 | 1.884 | 0.171 |
| Bachacke | 14 | 77.8 | 11 | 66.7 | 0.271 | 0.600 |
| Lower abdominal pain | 4 | 22.2 | 3 | 20.0 | FE | 1.000 |
| •Go to obstetrician: | | | | | | |
| Yes | 10 | 55.6 | 14 | 87.5 | FE | 0.063 |
| No | 8 | 44.4 | 2 | 12.5 | | |
| #If no, the method used to treatment: | | | | | | |
| Vaginal washing using petadine | 3 | 37.5 | 0 | 0 | 3.754 | 0.153 |
| vaginal douche with vinegar | 3 | 37.5 | 0 | 0 | | |
| No treatment | 2 | 25.0 | 2 | 100.0 | | |
| If yes, the prescribed treatment by obstetrician: | | | | | | |
| Vaginal douches | 5 | 50.0 | 8 | 57.1 | FE | 0.527 |
| Vaginal suppositories | 10 | 100 | 11 | 78.6 | FE | 0.024* |
| Oral tablets | 3 | 30.0 | 2 | 14.3 | FE | 1.000 |
| Local ointment | 7 | 70.0 | 6 | 42.8 | FE | 1.000 |
| Antibiotic | 1 | 10.0 | 2 | 14.3 | FE | 1.629 |
| •The effect of previous medical treatment prescribed by the doctor on the symptoms of vaginal infection: | | | | | | |
| Not effective | 2 | 20.0 | 7 | 50.0 | FE | 0.210 |
| Some effectiveness | 8 | 80.0 | 7 | 50.0 | | |
| Completely effective | 0 | 0 | 0 | 0 | | |

#More than one answer was chosen Group I= Pregnant women used vaginal suppositories composed of peppermint oil and sugar. Group II=Pregnant women used placebo vaginal suppositories.

sparity from 1-3 were 50% and 60% respectively with no significant differences. The table also shows that the third trimester gestational age of group I and group II were 43.3% and 36.7% respectively with no significant differences.

Table (3): shows the previous history of vulvo-vaginal candidiasis of the studied pregnant women. the table shows that the highest percents of group I (60%) had Previous complain of vulvo-vaginal candidiasis, 55.6% of them go to Obstetrician, 80% of them had some effectiveness of the previous medical treatment. the table shows that more than the half of group II (53.3%) had previous complain of vulvo-vaginal candidiasis, 87.5% of them go to Obstetrician, 50% of them had some effectiveness of the previous medical treatment with non significant differences between both groups.

Table (4): shows the symptoms of the present vulvo-vaginal candidiasis (VVC) reported by the studied pregnant women before and after treatment. The table shows that there was significant improvement regarding the symptoms of the present VVC in group I, compared to group II. The table also represents that the differences was statistically significant between the group I and group II regarding the presence of vaginal discharge, vaginal itching, dysurea, dyspareunia and Bachacke (P < 0.0001, < 0.0001, <0.030, < 0.0001 and < 0.068 respectively)

Table 4. Symptoms of the present vulvo-vaginal candidiasis reported by the studied pregnant women before and after treatment (n=60)

| Symptoms of vulvo-vaginal candidiasis | Group I (n=30) | | | | χ^2 P | Group II (n=30) | | | | χ^2 P GI vs GII | | |
|---------------------------------------|----------------|------|----------|------|------------|-----------------|------|----------|------|----------------------|-------|---------|
| | Before tt | | After tt | | | Before tt | | After tt | | | | |
| | n | % | n | % | | n | % | n | % | | | |
| ●Presence of vaginal discharge: | | | | | | | | | | | | |
| Yes | 30 | 100 | 9 | 30.0 | 29.30 | 30 | 100 | 28 | 93.3 | FE | 0.000 | 22.844 |
| No | 0 | 0 | 21 | 70.0 | 0.0001* | 0 | 0 | 2 | 6.7 | 0.491 | 1.000 | 0.0001* |
| If yes, amount of discharge: | | | | | | | | | | | | |
| Scanty | 4 | 13.3 | 18 | 60.0 | 14.494 | 6 | 20.0 | 4 | 13.3 | 0.487 | 0.581 | 14.800 |
| Small | 12 | 40.0 | 7 | 23.3 | 0.0001* | 10 | 33.3 | 11 | 36.7 | 0.786 | 0.747 | 0.001* |
| Excessive | 14 | 46.7 | 5 | 16.7 | | 14 | 46.7 | 15 | 50.0 | | | |
| ●Consistency of discharge: | | | | | | | | | | | | |
| -Normal vaginal discharge | 0 | 0 | 26 | 86.7 | 42.881 | 0 | 0 | 3 | 10.0 | 4.292 | 0.000 | 35.374 |
| -Cottage-cheese-like discharge | 30 | 100 | 4 | 13.3 | 0.0001* | 30 | 100 | 26 | 86.7 | 0.117 | 1.000 | 0.0001* |
| -Watery to thick in consistency | 0 | 0 | 0 | 0 | | 0 | 0 | 1 | 3.3 | | | |
| ●Smell: | | | | | | | | | | | | |
| Yes | 16 | 53.3 | 0 | 0 | 20.902 | 17 | 56.7 | 16 | 53.3 | 0.000 | 0.000 | 19.181 |
| No | 14 | 46.7 | 30 | 100 | 0.0001* | 13 | 43.3 | 14 | 46.7 | 1.000 | 1.000 | 0.0001* |
| If yes, nature of smell: | | | | | | | | | | | | |
| Un pleasant odor | 2 | 12.5 | 0 | 0 | - | 2 | 12.5 | 2 | 12.5 | 0.000 | 0.000 | - |
| Pungent odor | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 1.000 | 1.000 | - |
| Smell like cheese water | 14 | 87.5 | 0 | 0 | | 15 | 87.5 | 14 | 87.5 | | | |
| ●Vaginal itching | 29 | 96.7 | 2 | 6.7 | 45.120 | 30 | 100 | 26 | 86.7 | FE | 0.000 | 35.42 |
| | | | | | 0.0001* | | | | | 0.112 | 1.000 | 0.0001* |
| ●Dysurea | 15 | 50.0 | 6 | 21.4 | 4.695 | 17 | 56.7 | 15 | 50.0 | 0.077 | 0.077 | 4.695 |
| | | | | | 0.030* | | | | | 0.796 | 0.796 | 0.030* |
| ●Dyspareunia | 20 | 66.7 | 3 | 10.0 | 18.05 | 18 | 60.0 | 18 | 60.0 | 0.000 | FE | 19.655 |
| | | | | | 0.0001* | | | | | 1.000 | 0.091 | 0.0001* |
| ●Bachacke | 23 | 76.7 | 13 | 43.3 | 9.561 | 21 | 70.0 | 21 | 70.0 | 0.000 | 0.098 | 3.332 |
| | | | | | 0.002* | | | | | 1.000 | 0.770 | 0.068 |
| ●Lower abdominal pain | 5 | 16.7 | 3 | 10.0 | FE | 7 | 23.3 | 6 | 20.0 | 0.000 | 0.102 | FE |
| | | | | | 0.706 | | | | | 1.000 | 0.747 | 0.472 |

*Significant (P<0.05) Group I= Pregnant women used vaginal suppositories composed of peppermint oil and sugar. Group II=Pregnant women used placebo vaginal suppositories. FE=Fisher Exact test

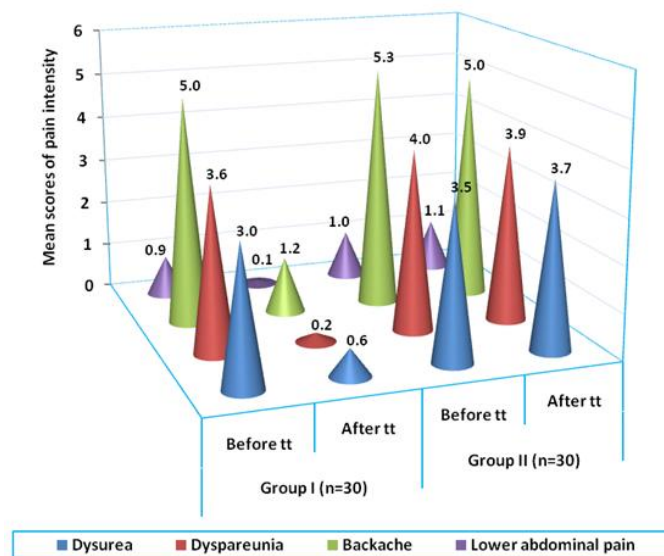


Figure 1. Mean scores of pain intensity of different symptoms assessed by Visual analogue scale among studied pregnant women with vulvo-vaginal candidiasis before and after treatment (n=60)

Table (5) and Figure 1: show the mean scores of pain intensity assessed by Visual analogue scale among the studied pregnant women with vulvo-vaginal candidiasis before and after treatment. As regard dysurea, dyspareunia, backache and lower abdominal pain, the mean scores of pain intensity were significantly higher among group I ($P < 0.0001$, < 0.0001 , < 0.0001 and $P < 0.031$ respectively) compared to that in group II. On the other hand, there was statistically significant difference between mean scores of pain intensity of both group I and II after treatment ($P < 0.0001$).

Figure 2. Represent the total pain intensity studied among pregnant women with vulvo-vaginal candidiasis before and after treatment (n=60)

Table (6) and (Figure 3 and 4) show the clinical examination findings of the present vulvo-vaginal candidiasis among the studied pregnant women before and after treatment. The table represents that there was significant improvement regarding the clinical signs of the present VVC in group I in comparison to group II. The table also shows that the differences were statistically significant between the group I and group II regarding presence of vaginal redness, vulvo-vaginal edema and the presence of vaginal discharge ($P < 0.0001$, < 0.001 and $P < 0.001$ respectively)

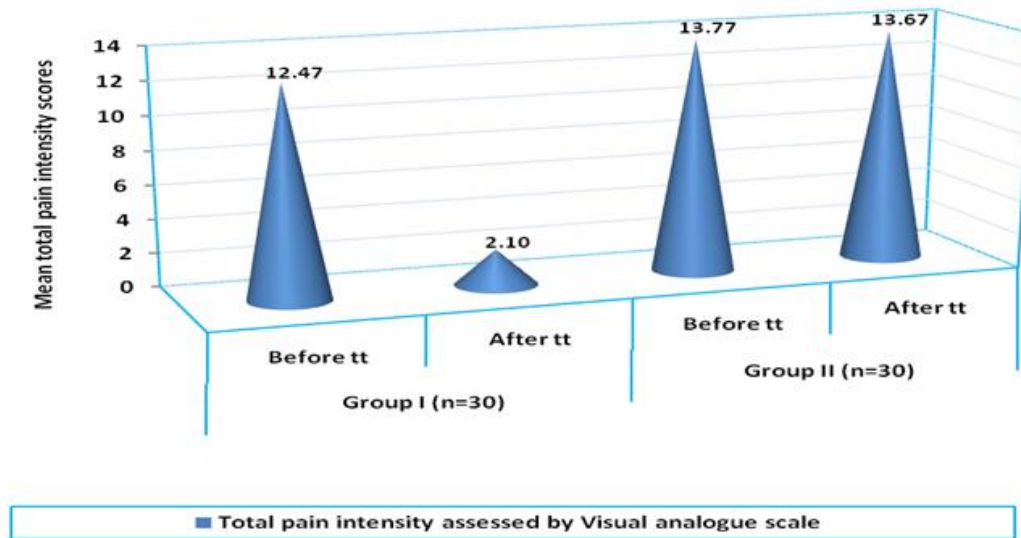


Figure 2. Mean scores of total pain intensity assessed by Visual analogue scale among studied pregnant women with vulvo-vaginal candidiasis before and after treatment (n=60)

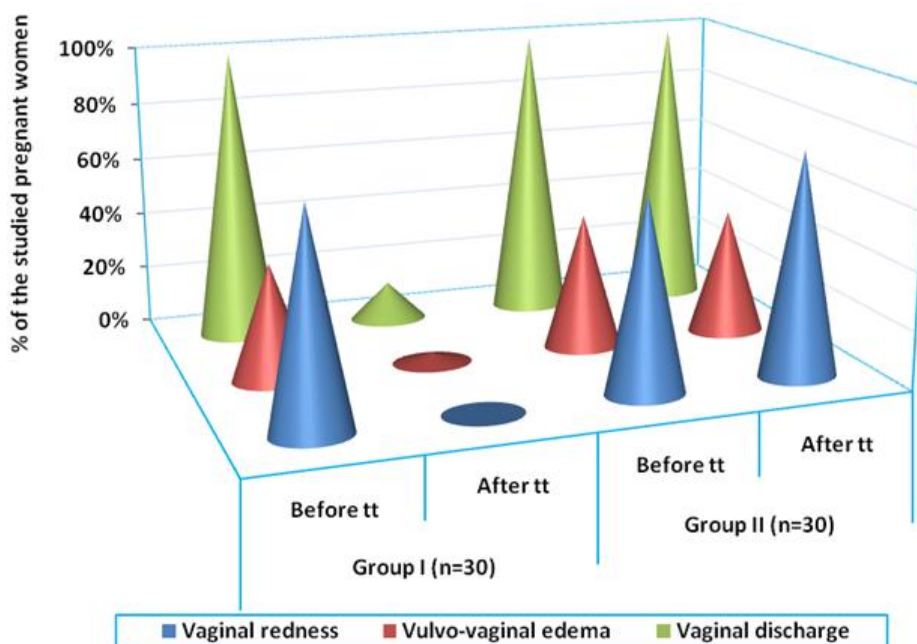


Figure 3. Clinical examination findings of the present vulvo-vaginal candidiasis among the studied pregnant women before and after treatment (n=60)

Table 5. Mean scores of pain intensity of different symptoms assessed by Visual analogue scale among studied pregnant women with vulvo-vaginal candidiasis before and after treatment (n=60)

| Visual analogue scale | Group I (n=30) | | Group II (n=30) | | ##Z value P GI vs GII | |
|-----------------------|----------------|-----------|-----------------|------------|-----------------------|----------|
| | Before tt | After tt | Before tt | After tt | Before tt | After tt |
| •Dysurea | | | | | | |
| Range | 0-8 | 0-2 | 0-8 | 0-8 | 0.559 | 5.547 |
| Mean±SD | 3.03±3.08 | 0.60±0.93 | 3.47±2.92 | 3.67±2.88 | 0.578 | 0.0001* |
| #Z value | 4.143 | | 0.267 | | | |
| P | 0.0001* | | 0.790 | | | |
| •Dyspareunia | | | | | | |
| Range | 0-9 | 0-3 | 0-9 | 0-9 | 0.492 | 6.994 |
| Mean±SD | 3.60±2.95 | 0.20±0.76 | 3.97±2.82 | 3.90±2.79 | 0.625 | 0.0001* |
| #Z value | 5.987 | | 0.092 | | | |
| P | 0.0001* | | 0.927 | | | |
| •Backache | | | | | | |
| Range | 0-10 | 0-4 | 0-10 | 0-10 | 0.431 | 6.296 |
| Mean±SD | 4.97±3.13 | 1.23±1.28 | 5.30±2.84 | 5.00±3.02 | 0.668 | 0.0001* |
| #Z value | 6.040 | | 0.396 | | | |
| P | 0.0001* | | 0.693 | | | |
| •Lower abdominal pain | | | | | | |
| Range | 0-7 | 0-2 | 0-7 | 0-7 | 0.321 | 2.501 |
| Mean±SD | 0.87±1.94 | 0.07±0.36 | 1.03±2.07 | 1.10±2.23 | 0.749 | 0.015* |
| #Z value | 2.217 | | 0.120 | | | |
| P | 0.031* | | 0.905 | | | |
| Total score | | | | | | |
| Range | 0-27 | 0-7 | 0-27 | 0-27 | 0.780 | 8.96 |
| Mean±SD | 12.47±7.02 | 2.10±2.12 | 13.77±5.83 | 13.67±6.74 | 0.439 | 0.0001* |
| #Z value | 7.741 | | 0.061 | | | |
| P | 0.0001* | | 0.951 | | | |

*Significant (P<0.05)

Group I= Pregnant women used vaginal suppositories composed of peppermint oil and sugar.

Group II=Pregnant women used placebo vaginal suppositories.

#Z value of Wilcoxon Signed Ranks Test

##Z value of Mann-Whitney U test

Table 6. Clinical examination findings of the present vulvo-vaginal candidiasis among the studied pregnant women before and after treatment (n=60)

| Clinical examination findings | Group I (n=30) | | | | χ^2 P | Group II (n=30) | | | | χ^2 P | χ^2 P GI vs GII | |
|------------------------------------|----------------|------|----------|------|------------|-----------------|------|----------|------|------------|----------------------|----------|
| | Before tt | | After tt | | | Before tt | | After tt | | | Before tt | After tt |
| | n | % | n | % | | n | % | n | % | | | |
| •Vaginal redness: | | | | | | | | | | | | |
| Present | 22 | 73.3 | 0 | 0 | 31.65 | 20 | 66.7 | 23 | 76.7 | 0.330 | 0.082 | 34.12 |
| Not present | 8 | 26.7 | 30 | 100 | 0.0001* | 10 | 33.3 | 7 | 23.3 | 0.567 | 0.778 | 0.0001* |
| •Vulvo-vaginal edema: | | | | | | | | | | | | |
| Present | 12 | 40.0 | 1 | 3.3 | 9.822 | 14 | 46.7 | 13 | 43.3 | 0.000 | 0.072 | 11.271 |
| Not present | 18 | 60.0 | 29 | 96.7 | 0.001* | 16 | 53.3 | 17 | 56.7 | 1.000 | 0.794 | 0.001* |
| • vaginal discharge: | | | | | | | | | | | | |
| Present | 30 | 100 | 4 | 13.3 | 42.424 | 30 | 100 | 30 | 100 | 0.000 | 0.000 | 42.424 |
| Not present | 0 | 0 | 26 | 86.7 | 0.0001* | 0 | 0 | 0 | 0 | 1.000 | 1.000 | 0.0001* |
| -Amount of vaginal discharge: | | | | | | | | | | | | |
| Normal | 0 | 0 | 26 | 86.7 | 54.671 | 0 | 0 | 0 | 0 | 0.198 | 1.097 | 53.144 |
| Mild | 2 | 6.7 | 4 | 13.3 | 0.0001* | 4 | 13.3 | 3 | 10.0 | 0.909 | 0.580 | 0.0001* |
| Moderate | 13 | 43.3 | 0 | 0 | | 10 | 33.3 | 11 | 36.7 | | | |
| Excessive | 15 | 50.0 | 0 | 0 | | 16 | 53.3 | 16 | 53.3 | | | |
| -Color of vaginal discharge: | | | | | | | | | | | | |
| Normal | 0 | 0 | 26 | 86.7 | 42.424 | 0 | 0 | 3 | 10.0 | FE | 0.000 | 32.307 |
| White | 30 | 100 | 4 | 13.3 | 0.0001* | 30 | 100 | 27 | 90.0 | 237 | 1.000 | 0.0001* |
| -Consistency of vaginal discharge: | | | | | | | | | | | | |
| Normal | 0 | 0 | 26 | 86.7 | 42.424 | 0 | 0 | 3 | 10.0 | FE | 0.000 | 32.307 |
| Cottage-cheese-like discharge | 30 | 100 | 4 | 13.3 | 0.0001* | 30 | 100 | 27 | 90.0 | 237 | 1.000 | 0.0001* |

*Significant (P<0.05)

Group I= Pregnant women used vaginal suppositories composed of peppermint oil and sugar.

Group II=Pregnant women used placebo vaginal suppositories.

FE=Fisher Exact test

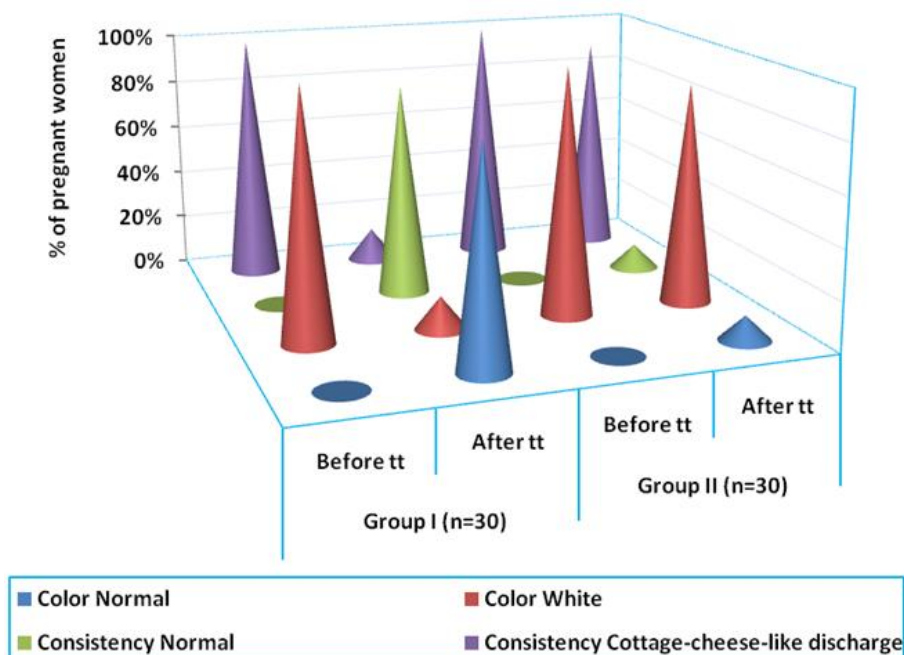


Figure 4. Clinical examination findings of vaginal discharge color and consistency of the present vulvo-vaginal candidiasis among the studied pregnant women before and after treatment (n=60)

Table 7. Patient's complain after using the formulating vaginal suppositories of the studied pregnant women with vulvo-vaginal candidiasis (n=60)

| Patient complain after using the formulating vaginal suppositories | Group I (n=30) | | Group II (n=30) | | χ^2 | P |
|--|----------------|------|-----------------|------|----------|---------|
| | n | % | n | % | | |
| •No complain | 28 | 93.3 | 5 | 16.7 | 35.650 | 0.0001* |
| •Burning sensation at the beginning time of suppository insertion | 2 | 6.7 | 24 | 80.0 | | |
| •Excessive watery vaginal discharge after suppository insertion | 0 | 0 | 1 | 3.3 | | |

*Significant (P<0.05)

Group I= Pregnant women used vaginal suppositories composed of peppermint oil and sugar.

Group II=Pregnant women used placebo vaginal suppositories.

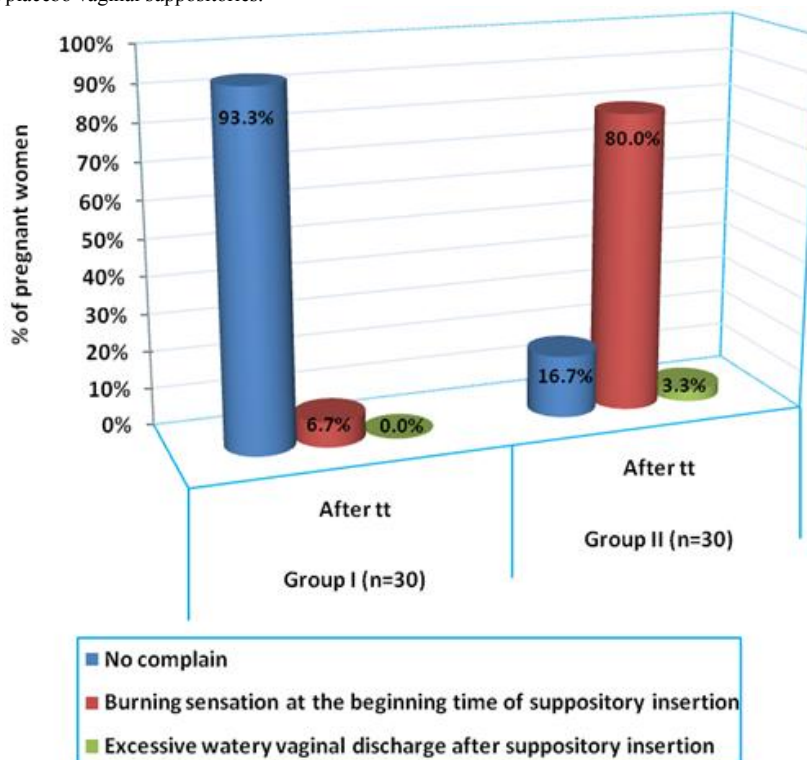


Figure 5. Patient complains after using the formulating vaginal suppositories of the studied pregnant women with vulvo-vaginal candidiasis (n=60)

Table (7) and Figure 5, show the patient's complain after using the formulating vaginal suppositories of the studied pregnant women with vulvo-vaginal candidiasis. The table revealed that the majority of the studied sample of group I and the rest of the studied sample of group II (93.3% and 16.7% respectively) had complain after using the formulating vaginal suppositories. On the other hand, the majority of the studied sample of group II and the rest of the studied sample of group I (80% and 6.7% respectively) had burning sensation at the beginning time of suppository insertion

oil and sugar demonstrated the fungicidal activity of these compounds (The zones produced around the discs in diffusion assay were completely clear, an indication of potential fungicidal activity)

DISCUSSION

The prevalence of vulvo-vaginal candidiasis (VVC) among pregnant women is very high. (Oyewole *et al.*, 2013; Nwadioha *et al.*, 2010; Akah *et al.*, 2010; Njlon and Nsagha 2012;

Table 8. Mean inhibition zone diameter in three dishes by using different concentrations of sugar and peppermint oil

| Concentrations of sugar & peppermint oil (mg/ml) | Inhibition zone diameter in three dishes (Mm) | | | |
|--|---|--------|--------|------------|
| | Dish 1 | Dish 2 | Dish 3 | Mean±SD |
| 1000/ suppository | 30 | 29 | 30 | 29.67±0.58 |
| 750 | 28 | 26 | 27 | 27.00±1.00 |
| 600 | 20 | 18 | 21 | 19.67±1.53 |
| 500 | 12 | 12 | 11 | 11.67±0.58 |
| 400 | 10 | --- | 10 | 10.00±0.00 |
| F value | | | | 234.804 |
| P | | | | 0.0001* |

Sugar: oil = 2:1 / 10% sugar and 5% peppermint oil

500mg sugar 250 mg peppermint oil

*Significant (P<0.05)

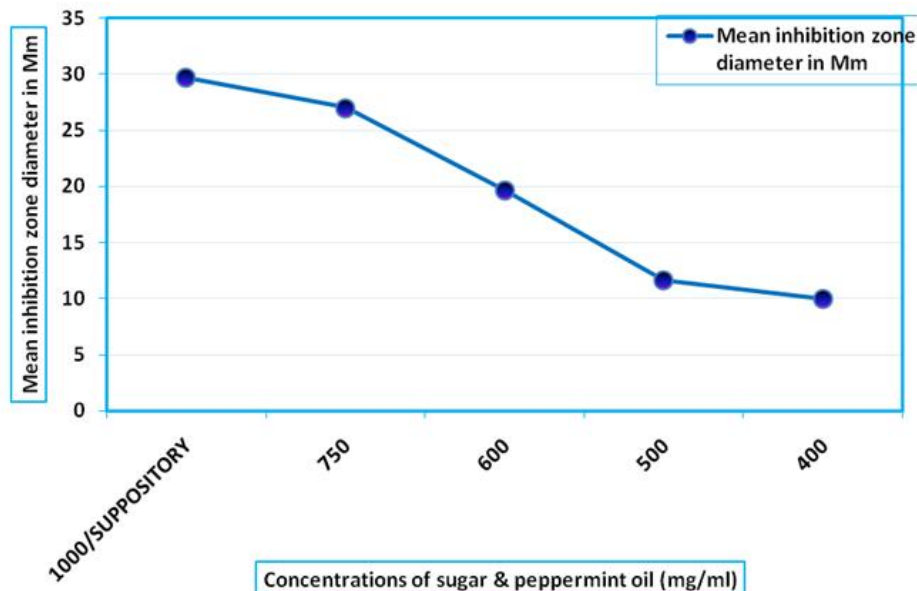


Figure 6. Mean inhibition zone diameter in three dishes by using different concentrations of sugar and peppermint oil

Table (8) and Figure (6), showed that peppermint oil and sugar (1:2) solution showed clear zones of inhibition (ZOI) which increased with increasing concentrations. At MIC (8%) showed a ZOI of 10 mm diameter which increased to 27 mm when the concentration was increased to (15%) against *C. albicans*. (Table 8) gives the values for the diameter of ZOI for all the test compounds in in different concentration at fixed ratio. There is an increase in (ZOI) with increasing the concentration it is clear that there is a significant difference until reaching the concentration of 20% where the plateau was reached so the most effective dose was as following: peppermint oil and sugar at a ratio of 1:2 was 10% sugar and 5% peppermint oil (15%). The zones of inhibition formed on solid agar media by exposing the *Candida* strains with MIC values of peppermint

Parveen and Munir 2008) A variety of systematic antifungal drugs are currently available to treat it , but there is risk for these drugs to penetrate the placental barrier and enter fetal cord blood leading to serious risks such as, fetal malformations, intrauterine growth retardation and preterm labor, so many of these drugs are contraindicated during pregnancy. (Moudgal and Sobel 2003) Peppermint oil is one of the most widely used safe essential oils that has antifungal, antibacterial and antimicrobial properties; also it has a soothing and cooling effect on skin inflammation. (Miller 2014) On the other hand, the sugar can help the water moves from the side with less sugar inside the cells to the side with more sugar out side the cells to dilute the solution there that is the process of osmosis. (Kong 2014)

This study was conducted to determine the effect of vaginal suppository composed of peppermint oil and sugar as natural and safe components were formulated by the researchers due to their effects on the signs and symptoms of VVC among pregnant women. This study revealed that the highest incidence of the signs and symptoms of vulvo-vaginal candidiasis was observed among the age group ranged from 20-40 years in group I and from 20-42 years in group II, this result was supported with Willacy *et al.* (2011) who reported that a peak of VVC between the age group of 20-40 years, (Willacy *et al.*, 2011) however it disagreed with Oyewole *et al.* (2013) who assessed the prevalence of vaginal candidiasis among pregnant women attending federal university of technology, Minna, Nigeria, Bosso Clinic, who found that the highest incidence of VVC was observed among the age group ranged from 21-30 years, followed by the age range of 31-40 years. (Oyewole *et al.*, 2013) This result may be due to high sexual activity, poor personal hygiene, the use of contraceptives, inadequate knowledge, limited diagnostic facilities, poor dietary habits, shortage of effective treatment, increased levels of estrogens and corticoids, wearing of tight-fitting synthetic underclothing, prolonged use of antibiotics which kill the good and beneficial bacteria among this age group. (Willacy and Jackson 2011; Lamia *et al.*, 2004) This result also was contradicted with Fardiazar *et al.* (2012) who investigate the vulvovaginal candidiasis recurrence during pregnancy, they found that there was no statistically significant relationship between women's age and the recurrence rate of VVC during pregnancy. (Fardiazar and Ronaci 2012)

It is obvious from the present study that there was agreement between the both studied groups, so there were no statistically significant differences between them regarding the socio-demographic characteristics, body mass index (BMI) and their obstetrical history. The present study denoted that three fifths of group I and nearly three quarters of group II who suffered from signs and symptoms of VVC were multigravida. This result was in agreement with the study of Aslam *et al.* (2008) who reported that the high percent of pregnant women suffered from VVC were multigravida (Aslam *et al.*, 2008), this high prevalence among the multigravida women may be due to the previous use of contraceptive methods. (Nwadioha *et al.*, 2010) This result also was confirmed with the study done by Oyewole *et al.* (2013). Who revealed that the highest incidence of candidiasis was among multigravida. (Oyewole *et al.*, 2013) on the other hand the result of the present study disagreement with the study done by Fardiazar *et al.* (2012), who found there was no positive relationship between gravidity and the recurrent rate of vaginitis during pregnancy time. (Fardiazar and Ronaci 2012)

As regard the gestational age of the both studied groups, the present study revealed that the highest percent of group I and group II belonged to the third trimester of pregnancy. This result was in agreement with the result of Fardiazar Z *et al.*, (2012), who found that the most of their studied women experienced recurrence of VVC infection twice during the third trimester of pregnancy, (Fardiazar and Ronaci 2012) This result may be due to the high production or changes in the levels of sex hormones and deposition of glycogen in the vagina at that time. (Bankar *et al.*, 2012) However this result contradicted with Oyewole *et al.* (2013) who reported that the

highest occurrence of vulvo-vaginal Candidiasis was found in the second trimester followed by third trimester while the least occurrence was found in the first trimester and Aslam *et al.* (2008) who reported that pregnant women in the second trimester had the highest occurrence of Candida infection than first and third trimester. (Oyewole *et al.*, 2013; Aslam *et al.*, 2008)

The present study shows that there was some effectiveness and non of them had completely effectiveness for the previous medical treatment prescribed by the doctor among women who had previous complain from the signs and symptoms of VVC. However, there was highly significant improvement in the signs and symptoms of VVC among studied women of group I who used the formulating vaginal suppository composed of peppermint oil and sugar, compared to group II who used placebo vaginal suppository. The present study also shows that the majority of the studied women in group I had no complain from the using of suppository. This means that the formulating suppository composed of peppermint oil and sugar was effective to treat the signs and symptoms of VVC.

Conclusion and Recommendation

It is concluded that vaginal suppositories composed of peppermint oil and sugar is an effective antifungal agent which can be used for treatment of vulvo-vaginal candidiasis during pregnancy without causing side effects. Further studies are needed to support the present study results regarding the effectiveness of those suppositories on the other types of vaginal infection.

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