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Bioapigyn® Ointment of Pelvic Muscle Tonus Versus Pelvic Floor Muscle Training for the Treatment of Urinary Incontinence

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Abstract

Objective/Purpose: The purpose of this work was the assessment of the clinical efficacy and safety of Bioapigyn® vaginal ointment for pelvic muscle tonus compared to pelvic floor muscle training in alleviating the symptoms of stress, urge and mixed urinary incontinence and vulvo-vaginal disorders in child-bearing and menopausal & postmenopausal women. **Materials and Methods:** The experimental group consisted of 66 women was treated 28 days with Bioapigyn® ointment for pelvic muscle tonus (2.5 mL/day). The control group also consisted of 66 participants was subjected to pelvic floor muscle training during 28 days (five times a day). ICIQ-UI SF score, the residual urine volume, perineometry, the total score of vulvo-vaginal symptoms and vaginal pH were determined before and after the treatment or training. **Results:** Following the treatment with Bioapigyn® ointment ICIQ-UI-SF score decreased 54.9%, perineometry parameters increased between 31.5 and 34.3%, residual urine decreased for 76.9% and vaginal pH for 14.2%. All the symptoms of vulvo-vaginal disorders disappeared completely in all participants. The control group showed no changes in vaginal pH or the improvement concerning the vulvo-vaginal complaints. ICIQ- UI-SF score decreased for 4.3%, residual urine volume for 9.1% while perineometry parameters increased between 4.3 and 8.3%. **Conclusion/Discussion:** Bioapigyn® vaginal ointment for pelvic muscle tonus alleviate the symptoms of incontinence by tightening and firming of the smooth muscles of the pelvic floor thanks to the ingredients with smooth muscles contraction/relaxation and astringent properties. Low pH, high osmolarity, viscosity, greasiness and coating effect of the ointment eradicated vulvo-vaginal complaints.

Key Words: Urinary Incontinence, Vulvo-Vaginal Disorders, Honey, Herbal Macerates

1. Introduction

Urinary incontinence (UI) is the complaint of involuntary leakage of urine (Aoki et al., 2018). It is a common chronic condition that affects millions of persons around the world (Minassian et al., 2008). Although UI tend to increase with age it could affect people of all ages. Urinary incontinence occurs when the pressure within the bladder exceeds the total urethral resistance and urine flows involuntarily beyond the urinary sphincter

(Rovner and Wein, 2004; Aoki et al., 2018). It occurs as a result of abnormalities of the urethra or both that may result in either overfunction or underfunction of the bladder and/or urethra, resulting in the development of urinary incontinence.

Chronic UI is classified into five types: stress, urge, mixed, overflow and functional (Rovner and Wein, 2004; Khandelwal and Kistler, 2013; Aoki et al., 2018).

Stress urinary incontinence (SUI) is defined as the complaint of involuntary leakage during effort or exertion, or on sneezing or coughing (Luber, 2004; Khandelwal and Kistler, 2013; Aoki et al., 2018). It is characterized by loss of small amount of urine during physical activity or intra-abdominal pressure (coughing, sneezing jumping, lifting, exercise).

Urge urinary incontinence (UUI) is characterized by loss of urine preceded by a sudden and severe desire to pass urine in which patient typically loses urine on the way to the toilet (Khandelwal and Kistler, 2013). Unlike the physical changes associated with stress urinary incontinence, UUI involves physiological perturbations to bladder function. There are three main etiologies essential to the bladder that leads to urge incontinence: detrusor over activity, poor detrusor compliance and bladder hypersensitivity (Rovner and Wein, 2004; Khandelwal and Kistler, 2013; Aoki et al., 2018; Radzimińska et al., 2018).

Mixed UI presents the combination of stress and urge incontinence which is characterized by involuntary leakage associated with symptoms of urgency as well as loss of urine with exertion, effort, sneezing, or coughing (Rovner and Wein, 2004; Khandelwal and Kistler, 2013; Radzimińska et al., 2018). Although UI increases with age, its prevalence varies widely. The median prevalence of any type of UI in women based on 35 studies (Minassian et al., 2003) was 27.6% (with a range of 4.8–58.4%).

Among 1700 French women employed in academic hospital, 12.4% of them reported SUI (Peyrat et al., 2002). The pregnancy, particularly previous vaginal delivery and hysterectomy represented the significant risk factors. The prevalence of stress, urge, mixed, and any UI among 2,875 adult women were 23.7%, 9.9%, 14.5%, and 49.2%, respectively (Minassian et al., 2008). The obtained significant risk factors were age, ethnic background, weight, parity and hysterectomy.

Among 83,355 American nurses at the age range from 37 to 54 years 43% of them reported incontinence. Identified risk factors were age, race/ethnicity, body mass index, parity, smoking, type 2 diabetes mellitus, and hysterectomy (Danforth et al., 2006).

Among 20,000 Chinese women in the age range from 20 to 99 years the prevalence of UI was 30.9%. Among them 18.9%, 2.6%, and 9.4% were diagnosed with SUI, UUI and mixed incontinence, respectively (Zhu et al., 2009). The authors identified age, vaginal delivery, multiparty, alcohol consumption, central obesity, constipation, chronic pelvic pain, history of respiratory disease, gynecological events, pelvic surgery, and perimenopause and postmenopause status as the significant risk factors. Buchsbaum et al. (2002), estimated the prevalence of urinary incontinence among a group of nulliparous nuns which was app. 50%. Among them 30% had stress incontinence, 24% had urge incontinence, 35% had mixed incontinence, and 11% had urine loss unrelated to stress and urge. Identified covariates were BMI, multiple urinary tract infections, and depression.

Higher prevalence of UI (Brown et al., 1999) was obtained among postmenopausal women (56%). Luber (2004), reported the prevalence of SUI which ranged between 4% and 35% depending on the country with age, obesity, and smoking as the most significant risk factors. Nygaard and Heit (2004),

reported that SUI occurs at least weekly in one third of adult women.

A world wide survey conducted by McPhil (2004), revealed the highest percentage of women with SUI in UK (41%) and Canada (42%) and the lowest percentage were obtained in Spain (23%) while the mean value for all considered countries was 32%. Two-thirds of the symptomatic women were younger than 50 years.

The treatment approaches depending on the type of incontinence and its severity. The most common treatment approaches are various behavioral techniques, pelvic floor muscle training (PFMT), electrical stimulation, medical devices, medication, surgical procedures, laser treatment (Nygaard and Heit, 2004; Aoki et al., 2018). Better results were obtained by surgical and laser treatment. However, those methods were associated with more risk compared to the conventional treatment.

The purpose of this study was to assess the efficacy and safety of Bioapigyn® vaginal ointment for pelvic muscle tonus for the local treatment of incontinence and vulvo-vaginal disorders in adult female population in comparison of pelvic floor muscle training due to similar mode of action of those two approaches.

2. Materials and Method

2.1. Study design

The study was designed as prospective, randomized, controlled clinical trial. The study protocol was approved by the Ethics Committee of Findri Gustek Health Care Center with EudraCT number 2019-001053-23.

The investigator recruited the patients based on their medical history, following the predefined inclusion and exclusion criteria. In total, 132 patients were included of 160 patients screened. After the informed consent has been signed at Visit 1, Day 1 all the patients completed International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF), and subjected to the measurement of post voiding residual urine volume, perineometry (maximal and average pressure (mm Hg) and the mean duration of the contractions), vaginal pH and self-assessment of vulvo-vaginal complaints. The questionnaire data and the clinical study results were recorded in source data. The patients were subjected to semi-quantitative urine analysis and full gynaecological examination in order to exclude other disease and conditions.

Patients were included in the study only if they meet all of the following criteria: stress, urge or mixed urinary incontinence and vulvo-vaginal disorders; history of vaginal delivery; non-existence of other gynecological problems; negative urine test; no injuries and bleeding in the vaginal canal, introitus and vestibule; in the investigator's judgment, the patients should receive local treatment only; signed informed consent. The patients who met all the criteria were divided into experimental and control group by nurse based on the randomization code.

The patients of the experimental group were given two tubes of Bioapigyn® vaginal ointment for pelvic muscle tonus with an applicator and instructions for use. Control group was subjected to pelvic floor muscle training and received training instructions.

66 patients were treated with Bioapigyn® vaginal ointment for pelvic muscle tonus for 28 days. 2.5 mL of ointment was self-administered by the patients once daily, between 21:00 and 24:00 hours using appropriate applicator. The second group also consisted of 66 patients was scheduled to the pelvic floor muscle training for 28 days according to the instruction given by the nurse. In short the patient mast contracts the pelvic floor muscles, holding the contractions for 10 seconds and then relaxes for 10

seconds. It must be repeated ten times five times daily. The purpose of PFMT was to improve the pelvic floor muscle function. Follow-up period for both groups was from Day 29 to Day 60.

2.2. Description of investigational product

Bioapigyn® vaginal ointment for pelvic muscle tonus is homogeneous, greasy, viscous mass of characteristic herbal odor and olive green color with pH of 4.93, density of 0.9801 g/cm^3 and viscosity of 20732 cP. It consists of the following ingredients: honey; beeswax (*Cera flava*); glycerol; oil extracts of the plant species: areal parts of shepherd 's purse (*Capsella bursa-pastoris* L.), nettle leaves (*Urtica diodica* L.), oak bark (*Quercus robur* L.), sage leaves (*Salvia officinalis* L.), areal parts of yarrow (*Achillea millefolium* L.), lady's mantle leaves and steam (*Alchemilla vulgaris* L.), marigold flowers (*Calendula officinalis* L.), cammomile flowers (*Matricaria chamomilla* L.), plantain leaves (*Plantago major* L.), olive leaves (*Olea europaea* L.); essential oils: Australien tea tree (*Melaleuca alternifolia*), thyme (*Timus vulgaris* ct. thymol), oregano (*Origanum vulgare*). Detailed description of the ointment production was published previously (Oreščanin et al., 2018).

2.3. Statistical analysis

Statistical analysis was performed using STATISTICA 12.0 software package. The required sample size was calculated by Power analysis method. Considering the medium effect size (0.35), the power goal of 80% and the Type I error significance level of 0.05, the required sample size is 66 participants per group, amounting to the total of 132 participants. Frequencies and percentages were calculated for each categorical variable using frequency tables. Potential differences between the groups of categorical variable were determined using $\chi 2$ Test. Basic statistical parameters were determined for each continuous variable. The Shapiro-Wilk W-Test was used to determine the normality of distribution of continuous variables, while Levene's Test was used for homogeneity of variances. The t test was used to determine the difference between mean values of two groups with normally distributed continuous variables, while the analysis of variance and Newman-Keuls test were used for the assessment of difference among three and more groups. Before carrying out the above-mentioned statistical analyses of data, logarithmic transformation was used to deal with the variables which deviate from the normal distribution. To determine the dependence of the dependent variable on the chosen predictor variables, multiple regression analysis was applied. The p-value of less than 0.05 ($p<0.05$) will be considered statistically significant in all measurements.

3. Results

3.1. Description of the population

The control population ranged from 50 to 73 (56.8±6.2) years and experimental group from 34 to 80 years (58.7±9.3). T-test showed no significant difference ($t=1.4$; $p=0.153$) between these two groups. There was no significant difference ($t=0.2$; $p=0.665$) in the number of childbirth between control (2.1±0.9) and experimental group (2.0±1.1). Both groups showed similar mean values and standard deviations for body mass index (26.3±4.8 for experimental and 25.7±4.1 for the control group). There was no significant difference between those two values ($t=0.6$; $p=0.434$).

Among 66 participants of the experimental group 20 of them are reproductive age woman while 46 of them are either menopausal or postmenopausal woman. Similar distribution was also found in the control group consisted of 28 reproductive age participants and 38 of those of menopausal & postmenopausal status. According to the results of $\chi 2$ test there was no significant difference between the

percentages of either reproductive age or menopausal & postmenopausal woman between the experimental and control group.

3.2. Treatment efficiency of vulvo-vaginal disorders

Table 1 presents the basic statistical parameters and the results of t-test for the symptoms of vulvo-vaginal disorders like unpleasant odor, itching, burning, vaginal discharge, vaginal dryness and dyspareunia graded from 0 to 3 and total score of those complaints in the control group subjected to pelvic floor muscle training and experimental group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the training or treatment.

Table 1. Treatment efficiency of vulvo-vaginal disorders

Group	Stat. parameter	Odor		Itching		Burning	
		Initial	Final	Initial	Final	Initial	Final
Control	$\bar{X}$	0.4	0.4	1.3	1.3	1.5	1.5
	SD	0.7	0.7	0.7	0.7	0.5	0.5
	M	0.0	0.0	1.0	1.0	1.5	1.5
	Min.	0	0	0	0	1	1
	Max.	2	2	3	3	3	3
	t-test	t=0; p=1.000		t=0; p=1.000		t=0; p=1.000	
Experimental	$\bar{X}$	0.5	0.0	1.3	0.0	0.0	1.2
	SD	0.6	0.0	0.5	0.0	0.0	0.5
	M	0.0	0.0	1.0	0.0	0.0	1.0
	Min.	0	0	0	0	0	0
	Max.	2	0	3	0	0	2
	t-test	t=5.7; p<0.0000*		t=20.3; p<0.0000*		t=7.6; p<0.0000*	

Table 1. Continued.

Group	Stat. parameter	Vaginal		Vaginal dryness		Dyspareunia		Total score	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	$\bar{X}$	1.4	1.4	1.0	1.0	1.2	1.2	6.7	6.7
	SD	1.1	1.1	0.9	0.9	1.0	1.0	1.9	1.9
	M	2.0	2.0	1.0	1.0	1.0	1.0	6.0	6.0
	Min.	0	0	0	0	0	0	3	3
	Max.	3	3	3	3	3	3	12	12
	t-test	t=0; p=1.000		t=0; p=1.000		t=0; p=1.000		t=0; p=1.000	
Experimental	$\bar{X}$	0.5	0.0	2.0	0.0	0.0	2.0	7.6	0.0
	SD	0.5	0.0	0.6	0.0	0.0	0.5	1.6	0.0
	M	0.0	0.0	2.0	0.0	0.0	2.0	7.0	0.0
	Min.	0	0	0	0	0	0	5	0
	Max.	2	0	3	0	0	3	11	0
	t-test	t=28.4; p<0.0000*		t=31.4; p<0.0000*		t=31.4; p<0.0000*		t=38.6; p<0.0000*	

The total score of vulvo-vaginal complaints in the control group ranged from 3 to 12 (6.7±1.9). The number of the symptoms at baseline ranged from 3 to 5. The highest mean values were obtained for burning, vaginal discharge and itching. Expectedly, following the four weeks of the training there was no reduction in the number or severity of the symptoms since there was no concomitant treatment of vulvo-vaginal disorders during the course of PFMT.

In the experimental group the total score of vulvo-vaginal complaints ranged from 5 to 11 (7.6±1.6) at baseline. Quite opposite to the control group, all the patients treated four weeks with the Bioapigyn® ointment for pelvic muscle tonus showed no symptoms of vulvo-vaginal disorders following the treatment. During the application of the ointment and monitoring period none of the patient experienced side-effects or new symptoms of vulvo-vaginal disorders.

Table 1. Basic statistical parameters and the results of t-test for the symptoms of vulvo-vaginal disorders graded from 0 to 3 and total score of the patients with incontinence and vulvo-vaginal disorders in control (C) group subjected to pelvic floor muscle training and experimental (E) group treated with Bioapigyn ointment for pelvic muscle tonus before and after the treatment or training. X̄-mean; SD-standard deviation; M-median; *-significantly different at p<0.05.

Vaginal pH value (Table 2) in the control group at baseline ranged from 5 to 7 (5.8±0.6) and showed the same value following the pelvic floor muscle training.

The experimental group showed similar values at baseline ranging from 4.8 to 7.5 (6.0±0.7). Four weeks of the application of Bioapigyn® ointment for pelvic muscle tonus resulted in statistically significant (t=7.4; p<0.0000) decrease of vaginal pH ranging from 4.3 to 6.5 (5.2±0.6).

Table 2. Basic statistical parameters for vaginal pH of the patients

Stat. parameter	Control		Experimental	
	Initial	Final	Initial	Final
X̄	5.8	5.8	6.0	5.2
SD	0.6	0.6	0.7	0.6
M	5.8	5.8	6.0	5.0
Min.	5.0	5.0	4.8	4.3
Max.	7.0	7.0	7.5	6.5
t-test	t=0.0; p=1.0		t=7.4; p<0.0000*	

Table 2. Basic statistical parameters for vaginal pH of the patients with incontinence and vulvo-vaginal disorders in control group subjected to pelvic floor muscle training and experimental group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the treatment or training. X̄- mean; SD-standard deviation; M-median; *-significantly different at p<0.05.

3.3. The results of urinary incontinence treatment

Based on the results of the total values of International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF) and additional information obtained from the patients when included in the study it was determined that among 66 participants of the experimental group 39 of them suffered from stress UI, 17 from mixed UI and 10 from urge UI (Table 3).

Stress urinary incontinence also prevailed in the control group with 45 of 66 participants. 11 of them had urge and 10 mixed urinary incontinence. Obtained results were in agreement with previous research confirming the highest incidence of SUI, followed by mixed and UUI (Buchsbaum et al., 2002; Minassian et al., 2008; Zhu et al., 2009).

Table 3. Frequencies and percentages of the subject of experimental and control group with stress (SUI), urge (UUI) and mixed urinary incontinence.

Type of incontinence	Experimental		Control	
	N	%	N	%
SUI	39	59.1	45	68.2
UUI	10	15.2	11	16.7
Mixed	17	25.8	10	15.2

The severity of the symptoms in both groups were determined at baseline and after four weeks of the treatment or training on the bases of the total value of ICIQ-UI SF score and the results were presented in Table 4. At baseline among 66 participants of the experimental group, 9 of them experienced only mild symptoms, 29 moderate, 26 severe and 2 very severe symptoms. Among the participants of the control group 11 of them had slight, 27 moderate and 28 of them severe symptoms of UI.

Following the treatment with Bioapigyn® ointment for pelvic muscle tonus, none of the patients reported very severe symptoms of incontinence while 18 of them (27.3%) were completely dry. Complete disappearance of the symptoms was obtained in the patients with prevalently stress urinary incontinence with initial ICIQ-UI SF score ranging from 3 to 10.

The percentage of the patients with severe symptoms decreased from 39.4% to only 6.1%. Those results were in line with the previous research results following two weeks of the application of Bioapigyn® ointment for pelvic muscle tonus (Orescanin et al., 2018). After the treatment, 4.56% of the patients were completely dry while very severe symptoms decreased from 10.61% to 0 and severe symptoms from 42.42% to 25.75%. Better results obtained in the current study were the function of two times longer treatment period.

Table 4. Frequencies and percentages of the subject of experimental (E) and control (C) group before and after the treatment or training based on the severity of the symptoms of urinary incontinence.

Severity	Initial				Final			
	E		C		E		C	
	N	%	N	%	N	%	N	%
Non (0)	0	0	0	0	18	27.3	2	3.0
Slight (1-5)	9	13.6	11	16.7	22	33.3	13	19.7
Moderate (6-12)	29	43.9	27	40.9	22	33.3	26	39.4
Severe (13-18)	26	39.4	28	42.4	4	6.1	25	37.9
Very severe (19-21)	2	3.0	0	0	0	0	0	0

Some improvement was also obtained in the control group subjected to PFMT. Two patients were completely dry, 13 of them experienced slight, 26 moderate and 25 severe symptoms. Similar to the experimental group complete disappearance of the symptoms was obtained in the patients with stress urinary incontinence with initial ICIQ-UI SF score of 3. When comparing the treatment with Bioapigyn® ointment for pelvic muscle tonus with PFMT applied for the same time-period the ointment treatment was found superior compared to PFMT. The total ICIQ-UI-SF score in the control group (Table 5) at baseline ranged from 3 to 18 (10.8±4.3). Slight decrease was obtained following the training to 9.7±4.6. However, this decrease was not statistically significant ($t=1.4$; $p=0.1770$). In the previous study (Oreščanin

et al., 2018), following the six months of PFMT the mean value of ICIQ-UI SF score slightly decreased from 8.34±4.21 to 7.92±4.71, which was not statistically significant. In the experimental group the initial values of ICIQ-UI-SF score ranged from 3 to 21 (11±4.5). Those values decreased significantly following the treatment (t=7.87; p<0.0000) with the range from 0 to 17 while mean values and standard deviation decreased to 4.9±4.5.

Table 5. Basic statistical parameters for total ICIQ-UI-SF score of the patients with incontinence and vulvo-vaginal disorders in control group subjected to pelvic floor muscle training and experimental group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the treatment or training. $\bar{X}$- mean; SD-standard deviation; M-median; *- significantly different at p<0.05

Stat. parameter	Control		Experimental	
	Initial	Final	Initial	Final
$\bar{X}$	10.8	9.7	11.0	4.9
SD	4.3	4.6	4.5	4.3
M	10.5	9.5	12.0	5.0
Min.	3.0	0.0	3.0	0.0
Max.	18.0	18.0	21.0	17.0
t-test	t=1.4; p=0.1770		t=7.87; p<0.0000*	

The results of perineometry and post voiding residual urine volume before and following the training or treatment with the ointment were presented in Table 6.

In the control group the initial values of the maximum pressure ranged from 0 to 32 (18.5±10.9), the average pressure from 0 to 30 (15.1±9.7) and duration of pressure from 0 to 32 (15.5±10.9). Following the PFMT all three parameters increased slightly and reached the mean values and standard deviations of 19.3±11.4, 16.0±10.3 and 16.8±11.5 for maximum, average and duration of pressure, respectively. Based on the results of t-test none of these values showed statistically significant increase compared to the initial values. In the experimental group the initial values of the maximum pressure ranged from 0 to 28 (11.0±9.0), the average pressure from 0 to 24 (8.1±7.5) and duration of pressure from 0 to 32 (9.4±9.9). Following the treatment with Bioapigyn® ointment all three parameters increased and reached the mean values and standard deviations of 14.6±9.8, 10.7±8.4 and 12.7±10.4 for maximum, average and duration of pressure, respectively.

Based on the results of t-test all three parameters showed statistically significant increase compared to the initial values. Those results confirmed that Bioapigyn® ointment for pelvic muscle tonus increased significantly the pelvic muscle strength following four weeks of the application. Previous study (Oreščanin et al., 2018) also showed increase in the pelvic muscle strength parameters. However, due to two times shorter treatment period this increase was not statistically significant. The initial values of post voiding residual urine (Table 6) in the control group ranged from 0 to 30 mL (11.2±8.9 mL) and showed slight but not significant (p=0.5042) decrease to 10.1±8.4 mL (range 0 to 27.7 mL).

On the contrary, the experimental group showed statistically significant decrease (t=4.1; p<0.0000) of the volume of residual urine from 6.9±10.1 mL (range 0 to 61.3 mL) to 1.6±2.3 mL (range from 0 to 10.5 mL) which could be explained by increase of pelvic floor muscle performance increasing intravesical pressure high enough to enable empting the bladder almost completely. Only two weeks of the treatment with Bioapigyn® ointment (Orescanin et al., 2018) reduced significantly (p=0.0002) post voiding residual urine volume from 8.73±11.18 to 2.78±5.93 mL.

Table 6. Basic statistical parameters and the results of t-test for the perineometry and residual urine volume of the patients with incontinence and vulvo-vaginal disorders in control (C) group subjected to pelvic floor muscle training and experimental (E) group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the treatment or training. X- mean; SD-standard deviation; M-median; *-significantly different at p<0.05

Group	Stat. parameter	Max. pressure (mm Hg)		Average pressure (mm Hg)		Duration of pressure (s)		Residual urine (mL)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	$\bar{X}$	18.5	19.3	15.1	16.0	15.5	16.8	11.2	10.1
	SD	10.9	11.4	9.7	10.3	10.9	11.5	8.9	8.4
	M	21.0	22.0	15.5	16.5	14.5	15.5	9.7	9.3
	Min.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max.	32.0	33.0	30.0	30.0	32.0	34.0	30.3	27.7
	t-test	t=0.4; p=0.6851		t=0.5; p=0.5489		t=0.7;p=0.5113		t=0.7;p=0.5042	
Experimental	$\bar{X}$	11.0	14.6	8.1	10.7	9.4	12.7	6.9	1.6
	SD	9.0	9.8	7.5	8.4	9.2	10.4	10.1	2.3
	M	11.0	14.5	7.0	9.0	7.0	8.5	3.0	0.7
	Min.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max.	28.0	32.0	24.0	28.0	32.0	37.0	61.3	10.5
	t-test	t=2.21;p=0.0286*		t=2.04; p=0.0475*		t=2.09;t=0.0407*		t=4.1; p<0.0000*	

Table 7. The results of multiple regression analysis testing for the influence of selected predictor variables on the initial values of ICIQ-UI-SF score, perineometry (maximum and average pressure and duration of pressure) and post voiding residual urine. *-significantly different at p<0.05

Predictor variable	ICIQ-UI-SF		Maximum pressure		Average pressure		Duration of pressure		Residual urine volume	
	β	p	β	p	β	p	β	p	β	p
Age	0.45	0.0041*	0.49	0.0000*	0.46	0.0000*	0.38	0.0000*	0.19	0.0929
Menopause	0.56	0.0000*	0.27	0.0054*	0.28	0.0036*	0.44	0.0000*	0.32	0.0064*
No of birth	0.10	0.1879	0.00	0.9696	0.01	0.9327	0.02	0.6795	0.04	0.5954
BMI	0.13	0.0742	0.01	0.8944	0.03	0.6686	0.04	0.5337	0.02	0.7644
	R=0.63; p<0.0000*		R=0.70; p<0.0000*		R=0.70; p<0.0000*		R=0.76; p<0.0000*		R=0.48; p<0.0000*	

The influence of predictor variables (age, menopausal status, BMI and number of child-birth) on the initial values of the incontinence parameters of the total tested population was assessed by Multiple regression analysis (Table 7).

Multiple regression analysis confirmed, good to very good (R ranged from 0.48 to 0.76), statistically significant ($p<0.05$) influence of the predictor variables (Table 7) on the initial values of ICIQ-UI-SF score, perineometry and post voiding residual urine. Based on the results of beta coefficients and their significance level it seems that among four predictor variables only menopausal status and age exhibited statistically significant contribution. In the case of residual urine content the menopausal status was the only significant predictor variable.

Those results were in line with the previous research identifying age and menopause as the significant predictor of incontinence (Luber, 2004; Danforth et al., 2006; Minassian et al., 2008; Zhu et al., 2009). Those authors also identified obesity as significant predictor, however, our results failed to show any significant contribution. The reason for that lies in the fact that the population from the current study had an ideal or slightly elevated BMI.

According to the results of multiple regression it seems that menopausal status is the variable with the highest influence on all assessed incontinence parameters. ICIQ-UI-SF score and post-voiding residual urine volume were significantly higher in menopausal & postmenopausal participants compared to child-bearing age participants.

On the contrary, all three perineometry parameters that determine pelvic floor muscle strength were significantly lower in menopausal & postmenopausal woman compared to those of child- bearing age.

3.4. Quantification of the difference in the treatment efficiency between the experimental and control group

The results t-test showed significant difference for perineometry parameters and residual urine volume at baseline. Consequently, the direct comparison of the efficiency between the treatment and training was not possible. Since direct comparison of the mean values of selected variables wouldn't be appropriate approach due to differences in the initial values in two tested groups the percentage of the changes (decrease or increase) of the mean value of each variable following the treatment was calculated for both groups and the differences between the percentages were tested by χ^2 test (Table 8).

There was no decrease of the total score of vulvo-vaginal disorders and vaginal pH in the control group. ICIQ-UI-SF score decreased 9.8%, perineometry parameters increased between 4.3 and 8.3% while residual urine volume decreased for 9.1%. Better results for perineometry parameters were obtained following six months PFMT (Oreščanin et al., 2018) with increase ranging between 7.3 and 26.4%.

The experimental group showed better results for all tested variables compared to PFMT group. The application of the ointment resulted in 100% decrease of vulvo-vaginal disorders, 14.2% decrease in vaginal pH, 76.9% decrease in residual urine volume and 54.9% decrease in the mean value of ICIQ-UI-SF score. In the same time, perineometry parameters determining pelvic muscle strength increased between 31.5 and 34.3%. According to the results of χ^2 test the differences between two groups were statistically significant for all tested parameters.

Current results for the experimental group were significantly better compared to the previous study (Oreščanin et al., 2018) lasting only two weeks which resulted in

30.7% decrease of the mean value of ICIQ-UI score, 68.2% decrease of residual urine volume, 11.3% decrease of vaginal pH and between 25.3 and 31.7% increase of perineometry parameters. However, two weeks of the treatment were long enough for complete disappearance of vulvo-vaginal complaints.

Table 8. The percentage of decrease of the Total score of vulvo-vaginal disorders, vaginal pH, residual urine volume and ICIQ-UI-SF score and increase of perineometry parameters in the control and experimental group compared to initial value and the results of χ^2 between control and experimental group. *-significantly different at p<0.05

Variable	Control	Experimental	χ2	P
Total score-vulvo-vaginal disorders	0.0	100.0	128.0	<0.0001*
Vaginal pH	0.0	14.2	8.1	0.0045*
ICIQ-UI-SF score	9.8	54.9	28.6	<0.0001*
Maximum pressure (mm Hg)	4.3	33.1	16.2	0.0001*
Average pressure (mm Hg)	6.3	31.5	12.1	0.0005*
Duration of pressure (s)	8.3	34.3	11.8	0.0006*
Residual urine (mL)	9.1	76.9	59.2	<0.0001*

3.5. Clinical efficiency and treatment rating by the patients

Following the treatment with Bioapigyn® ointment for pelvic muscle tonus 25.8% of the participant of the experimental and 3% of the control subjects were completely dry which was recorded as clinical cure. Clinical improvement was found in 74.2% participants of the experimental and 97% of the control group.

Both approaches have received good ratings by the patients. Treatment with Bioapigyn ointment showed a significantly better rating (4.9±0.3) compared to PFMT (4.4.±0.6) which was expected due to better performance of the ointment compared to PFMT.

3.6. Clinical safety

At Visit II the respondents using medical device are asked by the principal investigator if they experienced any side effects or adverse reaction after the first application of the ointment as well as throughout the course of the study. Furthermore, a complete gynaecological examination was performed to determine the possible occurrence of adverse reactions (irritation of the vulvo-vaginal area, allergic reaction), worsening of the existing or the occurrence of new symptoms. Patients were asked to describe in their own words the feeling after the application of the ointment.

Patients described a slight feeling of tightening and contraction in the vaginal area. After 20 to 30 minutes following the application of the ointment, they were able to empty the bladder completely. Patients with vaginal dryness and accompanying symptoms (itching, burning, and pain) after a week of administration experienced a comfortable feeling of vaginal moisture whiles the symptoms of itching and burning has disappeared. None of the patients reported severe burning or itching in the vulva-vaginal area, nor the appearance of an allergic reaction or the exacerbation of the existing symptoms or the appearance of new symptoms. The gynaecologic examination did not show any signs of irritation or worsening

of the vulvo-vaginal disorders. Just opposite, the examination confirmed the recovery of the vaginal mucosa in menopausal and postmenopausal women. There was no increase of vaginal pH in none of the subjects. On the contrary in majority of women vaginal pH was reduced significantly and only in few of them remained the same. All the patients confirmed that they could keep the urine significantly longer, the number of urination during the night decreased, patients could empty the bladder better compared to the baseline. The measurement of the perineometry parameters confirmed slight to significant enhancement of the pelvic floor muscle strength in all participants. In all patients who had residual urine at the first visit, its volume was reduced at the second visit. Based on the above facts obtained from the patients or by direct examination and measurement it is possible to conclude that the medical device does not cause any adverse effects and is safe for vaginal administration in the dose of 2.5 mL per day for up to 28 days.

4. Discussion and Conclusions

Conducted study confirmed the efficiency of Bioapigyn® vaginal ointment for pelvic muscle tonus in alleviating urinary incontinence in women. The ointment decreased the total value of ICIQ-UI-SF score in all 66 subjects. This significant improvement was the consequence of the strengthening of the pelvic floor muscles which was quantitatively confirmed by increasing in the values of perineometry parameters (maximum pressure, average pressure, duration of the pressure) and reducing the volume of post voiding residual urine.

Consequently, this study has confirmed that the main mode of action of the ointment is physico-mechanical by causing the contraction and relaxation of the smooth muscles of the pelvic floor similar to PFMT or electrical stimulation. However, compared to the PFMT conducted during the same time period the ointment showed significantly better results considering all measured parameters.

Although, there are no published data on human studies considering the influence of a single ingredient on the contraction of smooth muscles, the in vitro results or those obtained on the animal model have confirmed that the plants *Capsella bursa-pastoris* and *Urtica diodice* (Grosso, 2011; Al-Snafi, 2015) induce smooth muscle contraction/relaxation activity. Moreover, smooth muscle contraction could be caused by the astringent property of the plants such as *Quercus robur, Achillea millefolium, Salvia officinalis, Olea europaea, Plantago major.* Smooth muscle contraction/relaxation stimulated by the ointment ingredients resulted in the tightening and firming of the smooth muscles of the pelvic floor and consequently, reduced the symptoms of incontinence significantly especially in perimenopausal and menopausal women (Oreščanin and Findri Gustek, 2016, Oreščanin et al., 2018).

Besides, product is also indicated for alleviations of vulvo-vaginal disorders that are often associated with incontinence in perimenopausal and menopausal women. Disappearance of all vulvo-vaginal disorders could be explained physical parameters like low pH, high osmolarity, high viscosity and greasiness, emollient as well as low water activity of Bioapigyn® ointment for pelvic muscle tonus leading to eradication of the symptoms of vulvo-vaginal disorders due to: the creation of unfavourable conditions for the growth, adhesion and multiplication of the pathogens; the creation of the protective coating on the vaginal mucosa, enabling its recovery and preventing further irritation; alleviation of the vaginal dryness due to the presence of the humectants; preventing pain during sexual intercourse due to its lubricating and coating effect.

Conflict of Interest

The authors have declared that they have no conflict of interest.

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