(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),

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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