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Effect of bee venom phonophoresis in obese polycystic ovarian women: A Single Blind Randomized Controlled Trial

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex disorder that adversely affect fertility. The purpose of this study was to determine the effect of bee venom (BV) phonophoresis in the treatment of obese PCOS women. Forty-six obese PCOS women with age range, 18- 39 years and body mass index 29- 43 kg/m^2 were recruited. Participants were assigned randomly into two equal groups; group A, 23 PCOS patients received phonophoresis with BV topical application in addition to low caloric diet (1200-1400 kcal /day) and group B (control group), 23 PCOS patients followed low caloric diet (1200-1400 kcal /day) and sham ultrasound sessions with plain gel without BV. Assessment was done by biochemical analysis of luteinizing hormone (LH), follicle stimulating hormone (FSH), LH/FSH ratio, and progesterone (P4) levels, measured before, after 7 weeks, and after 14 weeks of treatment. Forty-six obese PCOS women (group A n=23; group B n=23) were randomized and analyzed. Comparison between groups revealed that there was no significance difference between both groups after 7 weeks and after 14 weeks of treatment except for P4 that showed a significant increase in group A after 7 weeks of treatment and marked decrease in LH and LH/FSH ratio in the middle of treatment at group A. BV phonophoresis with diet and physical activity have beneficial effect in the treatment of obese PCOS women.

INTRODUCTION

Polycystic ovary syndrome is an endocrine disorder with reproductive dysfunction and complex clinical symptoms that required multiple treatment approach. It increases the incidence of infertility and may increase the risk of cardiovascular diseases and endometrial cancer (Ndefo *et al*., 2013; Zhang *et al*., 2017). PCOS affects 6 to 15% of reproductive age women worldwide (Kamalanathan *et al*., 2013).

Women with PCOS commonly complain of hirsutism, acne, insulin resistance, irregular menses and infertility related to anovulation (Katsikis *et al*., 2006; Messini *et al*., 2015). PCOS is considered a low grade inflammatory disease (Kelly *et al*., 2001; Repaci *et al*., 2011). Almost 50% of women with PCOS have android obesity, this type of obesity is very difficult during treatment and plays an important role in the treatment of PCO, reduction of weight by diet and exercise are the first treatment approach that could be considered in obese PCOS patients, but need long time and efforts in dealing with android obesity (Messini *et al*., 2016; Tolino *et al*., 2005). The second line of treatment of PCO is the induction of ovulation, which occur either medically or surgically, it started by medical treatment using antiestrogens and gonadotropins but it has many side effects as nausea, vomiting, headache and fetal malformation (Haseeb, 2002).

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Bee venom (BV) is a new safe approach for induction of ovulation (Kouchesfahani *et al*., 2010). BV decreased androgen and could alter the metabolic features of PCOS due its anti-inflammatory effects which could increase the chance of reproduction (Karimzadeh *et al*., 2012; Karimzadeh *et al*., 2013). BV injection into acupoint produced a significantly more potent anti-inflammatory and antinociceptive effect than injection into a non-acupoint (Kwon, 2002). The traditional therapy of BV as in the treatment of arthritis was direct stings by bees, this method induced pain and inflammation, or BV injection into acupoint is an invasive technique causing severe pain, also the bee stings have no control over the exact dose which may lead to poor patient compliance (Chen and Lariviere, 2010). For these causes the need of another method for the application of BV is very important. Kim and Kim, (2014) used ultrasound device for BV delivery in treating of soreness of the biceps brachii muscle, this technique (phonophoresis) was found to be an effective method for pain reduction and improvement in range of motion. Phonophoresis is a noninvasive method in which the ultrasound waves are used to enhance transdermal drug delivery (Cagnie *et al*., 2003). So, the aim of this study was to use the BV phonophoresis as a new noninvasive method in the treatment of obese PCOS without pain.

SUBJECTS AND METHODS

Study Design

The study was designed as a prospective, randomized, single-blind, pre–post-test, controlled trial. Ethical approval was obtained from the institutional review board at Faculty of physical therapy, Cairo University before study, commencement with number P.T.REC/012/00330. The study followed the Guidelines of Declaration of Helsinki on conduction of human research. The study started August 2015 and ended October, 2016

Participants

A convenient sample of 46 obese PCOS women was recruited from the gynecology department, Al-Sahel Hospital, Cairo University. They were enrolled and assessed for their eligibility to participate in the study. To be included in the study, the participants were diagnosis of PCOS based on ultrasonographic presentation of PCOS, their age ranged from 18 to 39 years and BMI ranges from 29 to 43 kg/m^2, with oligo/anovulation. The participants were excluded if they had metabolic disorders, endometriosis and chronic pelvic pain, athletes who had undergone intense physical activity and users of oral contraceptives, glucocorticoids, medical treatment of weight reduction, anti-androgens, ovulation induction agents, or any other drugs affecting hormone levels.

Randomization

Informed consent was obtained from each participant after explaining the nature, purpose, and benefits of the study, informing them of their right to refuse or withdraw at any time, and about the confidentiality of any obtained information. Anonymity was assured through coding of all data. The obese PCOS women were randomly assigned into two groups (group A and group B) by a blinded and an independent research assistant who opened sealed envelopes that contained a computer generated randomization card. No subjects dropped out of the study after randomization.

Interventions

Participants were randomly assigned into; Group A, 23 women who received low caloric diet and phonophoresis with topical BV. Group B, 23 women who followed the low caloric diet and received sham ultrasound sessions. All patients received detailed information about the role of weight loss in reproductive disorders and the benefits of lifestyle modification with particular regard to physical activity and diet. They were instructed to perform physical activity, even a 30 minutes' walk (Mulholland *et al*., 2011), especially during the day of the session. All subjects in both groups were instructed to follow low caloric diet (1200-1400 kcal /day) one week before and during the treatment (Mekawy and Omar, 2011).

Group A: The participants were tested for BV allergy; diluted BV, 0.05 ml, in normal saline (1 μg/ml) was injected intradermally into the forearm. If the tested lesion resulted in a wheal with a diameter of less than 10 mm and erythema with a diameter of less than 26.5 mm after 10 to 15 minutes, subjects could participate in this study (Kim and Kim, 2014; Hunt *et al*., 1976). Participants were treated with BV previously prepared gel (Nassify, 2014), as topical application using ultrasonic therapy instrument with frequency, 1-MHZ continuous mode twice weekly. First, each participant lied in prone position to receive phonophoresis on both point of urinary bladder(UB) 23 acupoints at the back, for total 10 minutes, then she was asked to lie in crock lying position to receive phonophoresis on the abdomen acupoint, Ren (6) and both point of Zigong, stomach (30) for total 20 minutes (Johansson and Stener-Victorin, 2013). Applying a total amount of about 30- 50 gram of BV gel each session, the patient received 0.6 mg up to maximum 1 mg BV (Kim *et al*., 2004). For the points, Ren (6) below the umbilicus and points of Stomach (30) with delicate skin and low fat, only intensity of a value, 0.5 W/cm^2, was used. Intensities used for both points of Zigong and both points of UB (23) (contained subcutaneous less fat) were 1.5 and 1 W/cm^2 respectively.

Group B: Participants followed a low caloric diet (1200-1400 kcal /day), they also received the sham ultrasound sessions using only plain gel without BV.

Outcome measures

The level LH, FSH, LH/FSH ratio, and P4 levels, were measured before, after 7 weeks and after 14 weeks of treatment, the blood samples were collected during the estimated 2nd or 3rd day of menstruation cycle for assay LH and FSH and at the estimated mid luteal phase for progesterone level assay.

Sample size and Statistical analysis

To avoid a type II error, a preliminary power analysis [power (1−α error P) = 0.85, α = 0.05, effect size = 0.92, with a two-tailed for a comparison of 2 independent groups] determined a sample size of 23 for each group in this study. This effect size was calculated according after a pilot study of 12 participants (6 in each group) considering P4 as a primary outcome. All statistical measures were performed using the Statistical Package for Social Science (SPSS) program version 22 for windows. The current test involved two independent variables. The first one was the (tested groups); between subject factor which had two levels (group A and group B). The second variable was the (measuring periods); within subject factor which had three levels (pre and post 1 of treatment, and post 2 of treatment). In addition, this test involved four tested dependent variables (LH, FSH, LH/FSH, and P4). Prior to final analysis, data were screened for normality assumption, and presence of extreme scores. This exploration was done as a pre-requisite for parametric calculation of the analysis of difference and analysis of relationship measures. Descriptive analysis using histograms with the normal distribution curve showed that the data were normally distributed and did not violate the parametric assumption for the LH, FSH, LH/FSH. As well as, normality test of data using the Shapiro-Wilk test was used, that reflect the data was normally distributed for LH, FSH, LH/FSH and not normally distributed for P4. So, 2x2 mixed design ANOVA was used to compare the LH, FSH, LH/FSH at different measuring periods. Also, Friedman (nonparametric alternative to the repeated measure ANOVA) was used to compare the P4 at different measuring periods and "Wilcoxon signed rank tests" was used as post hoc tests if Friedman test among three measuring periods is significant. As well as, non-parametric statistical tests in the form of Mann-Whitney U test was used to compare between both groups. The alpha level was set at 0.05.

RESULTS

A total of 50 obese PCOS women were eligible for inclusion, and 46 were randomized to study intervention (fig 1). (Group A, received diet, 1200-1400 kcal with BV phonophoresis and group B (control group) received only diet, 1200-1400 kcal) There were no statistically significant differences (P>0.05) between subjects in both groups concerning age, BMI (Table 1)
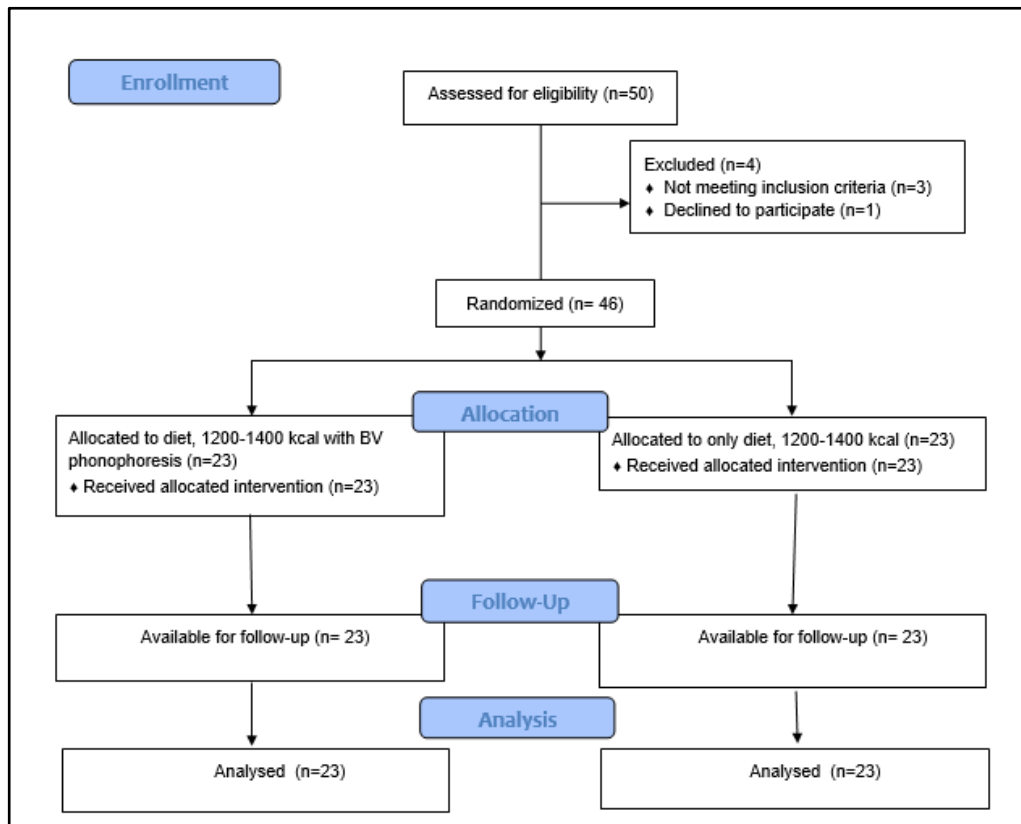

Fig. 1: Flow chart of the study.

Table 1: Mean values and SDs of age and BMI for all participants.

	Group A (N=23)	Group B (N=23)	p value
Age ± SD	26.0 ± 5.6	26.3 ± 6.5	0.86
BMI ± SD	34.2 ± 3.9	34.1 ± 4.4	0.9

Effect of BV phonophoresis on different variables

Statistical analysis using mixed design ANOVA analyzed forty-six patients assigned into two equal groups. Table (2) presents descriptive statistic (mean ± SD) and multiple pairwise comparison tests (Post hoc tests) for the LH, FSH, and LH/FSH. In the same context regarding within subject effect for LH, the multiple pair-wise comparison tests revealed that there was no significant difference (p >0.05) in the (Pre vs. post 1) at group B (control group) while there was a significant decrease (p <0.05) at group A (BV phonophoresis group). In addition, there was a significant decrease (p <0.05) in the (Pre vs. post 2) at both groups. Moreover, there was no significant difference (p >0.05) in the (Post 1 vs. post 2) at group A while there was a significant decrease (p <0.05) at group B.

Concerning FSH, there was no significant difference (p >0.05) in the (Pre vs. post 1) and (Pre vs. post 2) at both groups. While there was a significant increase (p <0.05) in the (Post 1 vs. post 2) at group A, but there was no significant difference (p >0.05) at group B.

Regarding LH/FSH, that there was no significant difference (p >0.05) in the (Pre vs. post 1) at group B while there was a significant decrease (p <0.05) at group A. In addition, there was a significant decrease (p <0.05) in the (Pre *vs*. post 2) at both groups. Additionally, there was no significant difference (p >0.05) in the (post 1 vs. post 2) at group A while there was a significant decrease (p <0.05) at group B. Regarding between subject effects, multiple pair-wise comparisons revealed that there was no significant difference of LH, FSH, and LH/FSH between both groups (p >0.05) at three measuring periods.

Table (3) present descriptive statistic (median and Interquartile Range) and comparison tests for P4 between both groups. The Friedman test revealed that there was a statistically significant difference in P4 among the three measuring periods (pre and post 1 of treatment, and post 2 of treatment) (p<0.05) for both groups. A Wilcoxon signed rank tests (Post hoc tests) revealed that there was a statistically significant increase in P4 in the post 2 of treatment compared with the pretreatment and post 1 of treatment (p<0.05) for both groups. In addition, there was a statistically significant increase in the P4 in the post 1 of treatment compared with the pre-treatment (p<0.05) at group A only. Considering the effect of the tested group (first independent variable) on P4, "Mann-Whitney U test" revealed that there was a significant increase in P4 at post 1 of treatment in favor to group A compared to group B (p<0.05).

Table 2: Descriptive statistics (mean ± SD) and comparison between patients with PCOS in pre, post 7 weeks (post 1) and post 14 weeks (post 2) of treatment for both groups.

	Group A (N=23)			Group B (N=23)		
	Pre-treatment	**Post 1**	**Post 2**	**Pre- treatment**	**Post 1**	**Post 2**
LH	8.13 ± 2.39	6.61 ± 2.08	6.31 ± 1.81	7.73 ± 2.39	7.47 ± 2.22	6.57 ± 2.44
FSH	5.99 ± 1.95	5.46 ± 1.49	6.36 ± 1.43	5.62 ± 1.71	5.48 ± 1.49	6.04 ± 1.38
LH/FSH	1.41 ± 0.37	1.23 ± 0.34	1.02 ± 0.34	1.42 ± 0.38	1.42 ± 0.51	1.11 ± 0.44
Within groups						
	Group A			**Group B**		
	Pre vs. post 1	**Pre vs. post 2**	**Post 1 vs. post 2**	**Pre vs. post 1**	**Pre vs. post 2**	**Post 1 vs. post 2**
LH	0.0001*	0.0001*	0.344	0.455	0.011*	0.007*
FSH	0.172	0.101	0.006*	0.722	0.063	0.077
LH/FSH	0.048*	0.0001*	0.096	1.00	0.005*	0.005*
Between groups						
Group A vs. Group B	**Pre-treatment**		**Post 1 treatment**		**Post 2 treatment**	
LH	0.579		0.182		0.683	
FSH	0.499		0.969		0.449	
LH/FSH	0.932		0.145		0.456	

*significant (p<0.05). SD: Standard Deviation.

Table 3: Descriptive statistics (median and Interquartile Range) and comparison tests between patients with PCOS in pre, post 7 weeks (post 1) and post 14 weeks (post 2) of treatment for both groups.

	Group A (N=23)			Group B (N=23)		
	Pre- treatment	**Post 1**	**Post 2**	**Pre-treatment**	**Post 1**	**Post 2**
P4	1.5 (2.1)	6.5 (8)	10 (9)	1.7 (1.3)	2 (2)	7 (9)
Friedman test (Within groups)						
P4	**X²-value**			**P-value**		
Group A	24.721			0.0001*		
Group B	15.438			0.0001*		
Wilcoxon signed rank tests (Post hoc tests)						
	Group A			**Group B**		
	Pre vs. post 1	**Pre vs. post 2**	**Post 1 vs. post 2**	**Pre vs. post 1**	**Pre vs. post 2**	**Post 1 vs. post 2**
P4	0.001*	0.0001*	0.0001*	0.065	0.0001*	0.0001*
Mann-Whitney U test (Between groups)						
Group A vs. group B	Pre treatment		Post 1 treatment		Post 2 treatment	
P4	0.956		0.012*		0.183	

*significant (p<0.05). IQR: Interquartile Range.

DISCUSSION

Polycystic ovary syndrome is a heterogeneous disorder consisting of clinical or biochemical hyperandrogenism with ovulatory dysfunction (Kamalanathan *et al.*, 2013). Endocrine disorders are the main clinical features of patients with PCOS, mainly manifested by increased level of LH, LH/FSH and testosterone hormones in blood, of which the reason may be the increased pituitary sensitivity to gonadotropin-releasing hormones, resulting in excessive LH secretion (Zhang *et al.* 2017). In the present study concerning LH and LH/FSH, there was a significant decrease (p <0.05) at group A (BV phonophoresis group) in the (Pre vs. post 1) and, there was a significant decrease (p <0.05) in the (Pre vs. post 2) at both groups, the results of current study agree with Ali *et al.* (2000)**,** who treated 15 PCO patients, not responding to medical treatment, by laparoscopic injection of BV, 0.1 ml into the ovarian stroma and observed a significant decrease in LH, androstenedione and testosterone. Ovulation occurred in 75% of the cases and the pregnancy rate was 50% in those who ovulated with no complications. The improvement in group A in the middle of the treatment could be explained by that ultrasound device either delivered BV to adipose tissue or to the systemic circulation could be help in fat catabolism and it could be suggested that the reduction in LH and LH/FSH was indirect outcome to the reduction of fat, as it was proved that LH/FSH ratio correlated significantly with total cholesterol (Ebrahimi-Mamaghani *et al.*, 2015). Also, this results can be explained by Karimzadeh *et al.*, (2012), who reported that BV treatment for 14 days in animal model with PCOS resulted in increasing lipolysis and decrease in thickness of the follicular theca layer. This agreed with Pouyanmanesh *et al.*, (2013) who reported that BV had a beneficial effect on PCOS probably through the reduction of triglycerides and low density lipoprotein levels. Also, it was also found that BV was effective in treating local abdominal obesity by decreasing the body weight and thickness of abdominal fat layer (Lim *et al.*, 2008)**.** Moreover, it was found that BV decreased levels of total cholesterol, triglycerides and certain anti-inflammatory cytokines in an atherosclerosis animal model (Lee *et al.*, 2010). In addition, there was a significant decrease in LH (p <0.05) at group B in the (Post 1 vs. post 2), this can have explained by the obese PCOS need time for weight reduction, this result came in accordance with Huber-Buchholz *et al.*, (1999) who found that 6-month diet and exercise program in cases of PCOS, decrease LH level. This was also consisted with Ndefo *et al.*, (2013), who reported that in obese PCOS weight reduction decrease androgen, LH and insulin levels, which regulating ovulation and improving the chance for pregnancy. Resumption of ovulatory cycles could occur after reduction of body weight (Crosignani *et al.*, 2003).

Concerning FSH, the results of the present study, the level of FSH significantly increased only in (post 2) treatment in group A compared to (post 1) treatment, while there was no significant difference (p >0.05) in the (Pre vs. post 1) and (Pre vs. post 2) at both groups. The conflicting results of FSH level could be hardly understood but could be explained as in PCOS patients, the level of FSH are within the normal range or slightly decreased (Zhang *et al.*, 2017; van Santbrink *et al.*, 1997), also can be explained by Palomba *et al.*, (2008), who stated that diet and exercise, in PCOS patients restored ovarian function without significant change in certain biochemical parameters as FSH.

The significant increase of P4 level in group A more than group B in the middle of the study let us suggest that BV might have ovulation induction properties, this suggestion agree with Ali *et al.*, (2003), who proved that BV played a role in maturation of follicular cells, so it could be considered as a new drug for induction of ovulation. Our results were also consistent with Karimzadeh *et al.* (2012) who found a significant increase in P4 level in PCOS rats treated with BV compared to untreated group. They attributed this to the ability of BV to adjust metabolic features and to its anti-inflammatory effects. They added that BV was able to increase the reproduction rate in PCOS rats through corpus luteum formation and reduction in ovary cysts. Moreover, Kouchesfahani *et al.* (2010) reported that corpus luteum was observed within BV treated ovary, in more than 70% of the cases investigated, indicating ovulation. The findings of the study may be limited by psychological and cultural issues. Although double blinding was not possible.

CONCLUSION

BV delivered by phonophoresis has beneficial effect in treatment of PCOS, it is non-invasive, simple method improving ovulation proved by increase progesterone level and could diminish the period recommended for weight reduction with diet and physical activity. Want to concern that in the short term, there is an improvement in all hormonal levels except for FSH only, which increased after 14 days, beside that on the level of the P4 there was a significant increase in both (7 days and 14 days) which suggest that with follow up there is further improvement.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ali AF, Fateen B, Ezzet A, Badawy H, Ramadan A, El-Tobge A. Laparoscopic intraovarian injection of bee venom in treatment of polycystic ovarian disease: a new modality. Obstet Gynecol, 2000; 95:252-56.

Ali AF, Mostafa M, Gaafar A, El-Shayeb S, El-Bashir Z. Bee venom promotes in vivo follicular development of immature rats. Fertil Steril, 2003; 80: 264-65.

Cagnie B, Vinck E, Rimbaut S, Vanderstraeten G. Phonophoresis versus Topical Application of Ketoprofen: Comparison between Tissue and Plasma Levels. P T, 2003; 83: 707-712.

Chen J, Lariviere WR. The nociceptive and anti-nociceptive effects of bee venom injection and therapy: a double-edged sword. Prog Neurobiol, 2010; 92: 151-183.

Crosignani PG Colombo M Vegetti W Edgardo, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate. Hum Reprod,2003; 18: 1928-1932.

Ebrahimi-Mamaghani M, Saghafi-Asl M, Pirouzpanah S, Aliasgharzadeh A, Aliashrafi S, Rezayi N, Mehrzad-Sadaghiani M. Association of Insulin Resistance with Lipid Profile, Metabolic Syndrome, and Hormonal Aberrations in Overweight or Obese Women with Polycystic Ovary Syndrome. J Health Popul Nutr, 2015; 33: 157–167.

Haseeb. 2002. F Basic obstetrics, 6th Ed., University Book Center, Cairo, PP 60-65.

Huber-Buchholz MM, Carey DG. Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab, 1999; 84: 1470-1474.

Hunt KJ, Valentine MD, Sobotka AK, Lichtenstein LM. Diagnosis of allergy to stinging insects by skin testing with Hymenoptera venoms. Ann Intern Med, 1976; 85: 56-59.

Johansson J, Stener-Victorin E. Polycystic Ovary Syndrome: Effect and Mechanisms of Acupuncture for Ovulation Induction. Evid Based Complement Alternat Med, 2013 Sep 2;2013.

Kamalanathan S, Sahoo JP, Sathyapalan T. Pregnancy in polycystic ovary syndrome. Indian J Endocrinol Metab,2013; 17: 37-43.

Karimzadeh L, Nabiuni M, Sheikholeslami A, Irian S. Bee venom treatment reduced C-reactive protein and improved follicle quality in a rat model of estradiol valerate-induced polycystic ovarian syndrome. J Venom Anim Toxins Incl Trop Dis, 2012; 18: 384-392.

Karimzadeh L, Nabiuni M, Kouchesfehani HM, Adham H, Bagheri A, Sheikholeslami A. Effect of bee venom on IL-6, COX-2 and VEGF levels in polycystic ovarian syndrome induced in Wistar rats by estradiol valerate. J Venom Anim Toxins Incl Trop Dis,2013; 19: 32.

Katsikis I, Kita M, Karkanaki A, Prapas N, Panidis D. Anovulation and ovulation induction. Hippokratia,2006; 10: 120–127.

Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N. Low Grade Chronic Inflammation in Women with Polycystic Ovarian Syndrome. J Clin Endocrinol Metab,2001; 86: 2453–2455.

Kim HW, Kwon YB, Ham TW, Roh DH, Yoon SY, Kang SY, Yang IS, Han HJ, Lee HJ, Beitz AJ, Lee JH. General pharmacological profiles of bee venom and its water soluble fractions in rodent models. J Vet Sci,2004; 5: 309-318.

Kim SK, Kim MC. The effect on delayed onset muscle soreness recovery for ultrasound with bee venom. J Phys Ther Sci,2014; 26: 1419-1421.

Kouchesfahani H, Nabiouni M, Adham H. Investigating the therapeutic effect of bee venom on polycystic ovarian syndrome in rats. Pejouhandeh, 2010; 15: 1-6.

Kwon YB, Lee HJ, Han HJ, Mar WC, Kang SK, Yoon OB, Beitz AJ, Lee JH. The water-soluble fraction of bee venom produces antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats. Life Sci,2002; 71: 191-204.

Lee WR, Kim SJ, Park JH, Kim KH, Chang YC, Park YY, Lee KG, Han SM, Yeo JH, Pak SC, Park KK. Bee venom reduces atherosclerotic lesion formation via anti-inflammatory mechanism. Am J Chin Med,2010; 38: 1077-1092.

Lim CS, Park WP, Jang SB, Choi YC, Kim KS, Kwon KR, Lee HC. Clinical studies of sweet bee venom to the effect of abdominal fat accumulation. J Pharmacopuncture, 2008;11(2):33-40.

Mekawy HS, Omar H. Efficacy of Exercise Augmented with Ultrasonic Cavitation on Female Sex Hormones and Ovulation in Obese Polycystic Ovarian Women. Bull Fac Ph Th Cairo Univ, 2011; 16: 63-71.

Messini CI, Anifandis G, Dafoulos K. Polycystic ovaries and obesity. Best Pract Res Clin Obstet Gynaecol, 2015; 29: 479-488.

Mulholland RS, Paul MD, Chalfoun C. Noninvasive body contouring with radiofrequency, ultrasound, cryolipolysis, and low-level laser therapy. Clin Plast Surg, 2011; 38: 503-520.

Nassify M. 2014. MSc Thesis. Al-Azhar University. In-Vitro and Therapeutic Effectiveness Evaluation of Some Preparations of Bee Venom.

Ndefo UA, Eaton A, Green MR. Polycystic ovary syndrome: a review of treatment options with focus on pharmacological approaches. P T, 2013; 38: 336-355.

Palomba S, Giallauria F, Falbo A, Russo T, Oppedisano R, Tolino A, Colao A, Vigorito C, Zullo F, Orio F. Structured exercise training programme versus hypocaloric hyperproteic diet in obese polycystic ovary syndrome patients with anovulatory infertility: a 24-week pilot study. Hum Reprod,2008; 23: 642-50.

Pouyanmanesh F, Nabiuni M, Nasri S, Nazari Z, Karimzadeh L. The effect of honey bee venom on levels of lipids and anti-mullerian hormone in a rat with polycystic ovarian syndrome. Feyz J, 2013; 17: 239-246.

Repaci A, Gambineri A, Pasquali R. The role of low-grade inflammation in the polycystic ovary syndrome. Molecular and Cellular Endocrinology, 2011; 335: 30–41.

Tolino A, Gambardella V, Caccavale C, D'Ettore A, Giannotti F, D'Antò V, De Falco CL. Evaluation of ovarian functionality after a dietary treatment in obese women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol, 2005; 119: 87-93.

Van Santbrink EJ, Hop WC, Fauser BC. Classification of normogonadotropic infertility: polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome. Fertil Steril, 1997; 67: 452-458.

Zhang J, Si Q, Li J. Therapeutic effects of metformin and clomiphene in combination with lifestyle intervention on infertility in women with obese polycystic ovary syndrome. Pak J Med Sci, 2017; 33: 8-12.