

# Analysis of Unani coded formulation on the hormonal parameters of patients with polycystic ovarian syndrome

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**Abstract:** The effect of Unani coded polyherbal formulations (*Picolin*) containing: 1) *Cinnamomum verum* (Bark), 2) *Glycyrrhiza glabra* (Root), 3) *Linum usitatissimum* (Seed) and 4) *Vitex agnus castus* (Seed) on the hormonal levels of polycystic ovarian syndrome (PCOS) patients (n =73) was determined. A randomized controlled multi-center trial was conducted on three groups that received either: 1) Capsule *Picolin* (n=25, 500 mg, two capsules thrice a day) and 2) Hydroalcoholic extract, tablet *Picolin-E* (n=23, 250 mg thrice a day) or 3) Metformin (n=25, 500 mg, twice a day) that continued for 12 weeks. The effect of capsule *Picolin* before and after treatment on serum insulin ( $14.8 \pm 1.8$  vs  $10.7 \pm 1.7 \mu\text{IU/mL}$ ) and prolactin ( $17.7 \pm 0.9$  vs  $8.5 \pm 1.1 \text{ng/mL}$ ) levels were significant. Likewise, the tablet *Picolin-E* also demonstrated significant effect on serum insulin ( $16.7 \pm 1.7$  vs  $9.3 \pm 0.1 \mu\text{IU/mL}$ ) and prolactin ( $18.5 \pm 1$  vs  $10 \pm 1.5 \text{ng/mL}$ ) levels. On the contrary in metformin treated group these parameters, serum insulin ( $17.4 \pm 1.6$  vs  $16.4 \pm 1.4 \mu\text{IU/mL}$ ) and prolactin ( $21.7 \pm 2.8$  vs  $21 \pm 2.8 \text{ng/mL}$ ) were non-significant. The improvement in the menstrual flow in the PCOS patients after treatment with either capsule *Picolin*, tablet *Picolin-E* or metformin were improved by 84%, 61% and 54%, respectively. Unani formulation were comparatively more effective than metformin. The treatment effectiveness was capsule *Picolin* > tablet *Picolin-E* > metformin.

**Keywords:** Polycystic ovarian syndrome, Unani medicine, management.

## INTRODUCTION

Polycystic ovarian syndrome (PCOS) (*Takyees al Mabayedh*) is a common endocrinal disorder affecting women during reproductive age (Kauffman *et al.*, 2008). It was described by Stein and Leventhal in 1935 as a syndrome of oligo-amenorrhea and polycystic ovaries which is variably accompanied by hirsutism, acne or obesity (Stein, 1935; Azziz and Adashi, 2016). In the PCOS patients, three common clinical features include ovulatory dysfunction, hyperandrogenism and polycystic ovaries (Rotterdam, 2004).

According to the criteria of National Institute of Health (NIH), Rotterdam and Androgen Excess Society and PCOS (AES-PCOS) the estimated prevalence of PCOS is 5-10%, 5-20% and 10-15%, respectively (Bozdag *et al.*, 2016). Among different population the variation in the prevalence of PCOS is most likely due to the influence of diverse ethnicity, lifestyle and obesity rate (Dargham *et al.*, 2017). The incidence of PCOS in Pakistan is alarming and has been reported to be 40.9% (Baqai *et al.*, 2010) which is the most prevalent gynecological disorder (55.41%) among women of premenopausal age (Zafar *et al.*, 2019).

Currently available medications for PCOS are symptom(s) oriented but have limitations because of its complex pathophysiology. The most preferred drug against PCOS is metformin which act as an insulin

sensitizer to improve insulin-glucose metabolism and anovulatory cycles in PCOS (Mehrabian *et al.*, 2016). However, its use is associated with various side effects such as bloating, diarrhea and nausea (Dodd *et al.*, 2019). Thus, it is imperative to explore various other alternatives to combat this gynecological problem.

According to Buqrat (Hippocrates) (460 B.C. to 375 B.C.) disease may occur due to imbalance of humors (*Akhlat*) referred as Hippocratic humoral theory (*Nazarya-e-akhlat*) (Qadeer, 2005) stating that every individual possesses a unique humoral mixture known as *mizaj* (Temperament) signifying a healthy state which is in equilibrium however, if it is imbalanced and not reverted to original state, disease condition may appear. The *mizaj* of an individual can be classified into 4 groups: 1) Sanguine (*Damwi*), hot and moist; 2) Phlegmatic (*Balghami*), cold and moist; 3) Choleric (*Safrawi*), hot and dry and 4) Melancholic (*Saudawi*) cold and dry. Thus, any imbalance of humor influences the *mizaj* of an individual and the Unani therapeutic approach has a capacity for its rectification (Ahmad, 1980). Although, the clinical features of PCOS is reflected by an increase of phlegmatic humor (*Khilt-e-balgham*), while Buqrat and Rabban Tabri proposed that liver dysfunction (*Sue Mizaj Kabid*) may also induce an abnormal production of phlegm leading to cyst formation in the ovaries, menstrual disturbance and obesity (Baghdadi, 2007). The resulting coldness (*Baroodat*) interrupts menstrual flow, ovum movement towards the uterus (Ibn-Sina, 2010) and also retards the growth of ovarian follicles (Majoosi, 1889), hence affecting the reproductive capacity of females

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causing infertility. In Unani medicine the treatment of PCOS is based on improvement of mal-temperament (*Sue-mizaj*) along with the use of emmenagogue drugs (*Mudire-haiz*) to facilitate menstrual flow (Khan, 2011; Hameed *et al.*, 2016).

## MATERIALS AND METHODS

### Preparation of the Unani formulation

The herbs used in the preparation of *Picolin* against PCOS were selected from Unani Pharmacopeia and published literature. Organoleptic evaluations of herbs were carried out in the Pharmacognosy Department, Karachi University and the voucher number for each herb was allocated: 1) *Cinnamomum verum* (Bark, CV-02-17), 2) *Glycyrrhiza glabra* (Root, CL-03-17), 3) *Linum usitatissimum* (Seed, LU-05-17) and 4) *Vitex agnus castus* (Seed, VA-10-17). The formulation was developed in two dosage forms: 1) Powdered form (capsule *Picolin*) and 2) Hydroalcoholic extract (tablet *Picolin*).

All the herbs were cleaned, examined for their impurities and adulteration prior to further processing as described below:

*Preparation of capsule (Picolin):* The herbs were weighed individually in a ratio of: a) *Cinnamomum verum* (Dar cheni/Cinnamon, 1kg), b) *Glycyrrhiza glabra* (Mulethi/Licorice, 1kg), c) *Linum usitatissimum* (Alsi/Flaxseed, 1.5kg) and d) *Vitex agnus castus* (Sambhalo/Chasteberry, 1.5kg). The total powder (*Safoof*, 5kg) of the herbs was used in capsule of size 0 and weight of individual capsule was 500 mg.

*Procedure of hydroalcoholic extract (Picolin-E Tablet):* Semi-crushed herbs (5 kg) were soaked in hydroalcoholic solvent (Ethanol 70%: Water 30%, 15L) in glass containers for 9 days. After every 3 days the solvent was collected, filtered carefully through the filter paper (Whatman number 19 $\mu$ m) to retain particles. The herbs were re-soaked in fresh hydroalcoholic solvent using ~15 liters of ethanol. The total filtrate (28L) obtained was transferred into the rotary flask to acquire extract. The final extract (1 kg) was collected and air dried with the help of air dryer at 50° C and stored in an airtight jar.

The wet granulation method was used for tablet preparation (Thapa *et al.*, 2019) initially, glucose (45%) was heated using water bath (100°C) until translucent semisolid mass was formed with water. The final extract (50%) and gum acacia (5%) were added gradually till the powder turned into a damp mass, passed through sieve (Number 20) and dried in an oven at a temperature of 600° C for 3hr, until granules were completely dried. The dried granules were sieved again (Number 20) and tablets were compressed using a single punch tableting machine (fig. 1).

### Tablet Friability test

The *Picolin-E* tablets (n=20) were weighed and placed in the friabilator at 25 revolutions /min. for 4min. The powder residues were removed and tablets were weighed again (Pharmacopeia, 2019).

Prior approval of both pre-clinical and clinical studies was obtained from the Hamdard Ethical Review Board, Hamdard University Karachi, reference number AEC-17-02 and conducted as follows:

### Animal studies

The toxicity study was carried following OECD 423 guidelines with a single dose exposure. Healthy 8-12 weeks old female albino mice, weighing about 15-25 g, were obtained from animal house of the Pakistan Council of Scientific and Industrial Research (PCSIR). The mice were randomly divided into 6 groups comprising 3 animals per group (n=18). All the animals were marked with a stain on the tail for identification. The animals were kept under standard animal housing conditions with controlled 12 h dark-light cycle, temperature (25 $\pm$ 2°C) and relative humidity (38%).

Before the start of experiment, the animals were acclimatized for a period of 5 days and provided with food and water *ad libidum* during the entire period of experiment. For acute toxicity test different concentrations (5, 50, 300 2000 and 5000mg/kg body weight) of the *Picolin* extract (n= 3/dose) were administered in a single dose orally while the control group received distilled water and the animals were kept under observation for 7 days.

### Antioxidant potential of Picolin-E

#### Determination of DPPH Radical Scavenging Activity

Free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as described earlier (Gulcin *et al.*, 2010). The solution of DPPH (0.3mM) was prepared in methanol. Sample (50 $\mu$ L, 5mg/ml) was mixed with DPPH (1 ml). The reaction mixture was incubated at 37°C for 30min. and the absorbance was measured (515nm) by spectrophotometer. Ascorbic acid was used as positive control. The percent radical scavenging activity was determined with reference to control.

$$\text{DPPH scavenging effect (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where  $A_c$  = Absorbance of Control,  $A_s$  = Absorbance of Sample

### Anti-inflammatory activity

Anti-inflammatory activity was conducted on albino rats using carrageenan induced paw edema method (Ravi *et al.*, 2009). Healthy adult albino rats (n=18) of both genders, weighing 200-250g were obtained from PCSIR. The rats were randomly divided into 3 groups (n=6 /group). Group-I (Control, distilled water, 1ml), Group- II (*Picolin-E*, 15mg/kg) and Group-III received standard drug diclofenac sodium (10mg/kg). The treatments were

given orally 30min. before the administration of carrageenan (0.1ml, 1%) injection in sub plantar region of the left hind paw of rat. Thereafter, paw volume was measured at 0, 30, 1, 2, 3 and 4 hour using plethysmometer (Nair *et al.*, 2012).

Percentage inhibition of paw edema was calculated using the formula:

$$(1 - V_t / V_c) \times 100$$

Where  $V_c$  and  $V_t$  represents paw volume of control and treated rats, respectively

### Clinical studies

Randomized clinical study was conducted at: 1) Shifa-ul-Mulk Memorial Hospital for Eastern Medicine, 2) Hamdard Matab Aram Bagh and 3) Naimat Begum Hamdard University Hospital, Karachi. After screening (n=153) PCOS of reproductively active age patients, subjects (n= 90) (table 1) were recruited between April to September 2018 fulfilling the inclusion criteria.

### Inclusion criteria

It included: 1) Females between 18-35 years of age, 2) Karachi residents, 3) No complications record of reproductive system, 4) PCOS diagnosed using Rotterdam criteria fulfilling any two of the following: (i) Menstrual irregularity or oligo-/anovulation, (ii) Hyperandrogenism (clinically and/or biochemically), (iii) Polycystic appearance on ultrasound.

### Exclusion criteria

These were: 1) Patients with severe adverse drug reactions, 2) Record of cardiovascular, diabetes, hepatic and neoplastic disorders or other concurrent medical illnesses, 3) Hormonal contraceptive used within 6 months, or anti-obesity drugs within 3 months of the study, 4) Irregular menstrual bleeding other than PCOS and 5) Positive pregnancy test.

All the patients were explained the purpose of the study and written consent was obtained. Demographic details and baseline clinical features at the time of enrolment were recorded in the clinical proforma. Each group comprised of n=30 patients which were randomly assigned the powdered form of the Unani formulation as *Picolin*, two capsules (500mg) three times daily, the hydroalcoholic extract of the Unani formulation referred as *Picolin-E*, one tablet, (250mg) three times daily. The metformin treated group received 500 mg tablet, twice a day. After 12 weeks (n=73) completed the treatment course and the number of subjects dropped out were 17/90 (18.8%).

The laboratory tests were performed after a 12 hour overnight fasting, during the follicular phase of the cycle or after at least 3 months of amenorrhea for proper analysis. *Mizaj* was assessed by the ten criteria (*Ajnas-e-Ashara*) of temperament assessment as described by *Ibn-*

*e-Sina* in Canon of Medicine. A questionnaire was prepared for scoring the subjective and objective parameters of each criterion for evaluation of *mizaj*.

The therapeutic assessment of the drug was based on the improvement in menstrual flow and endocrine parameters (Serum fasting insulin, prolactin, testosterone, sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH) and luteinizing hormone (LH)). The patients visited weekly for the first month followed by a monthly visit for their assessment. Each woman was also asked to report any side-effect during the treatment period.

## STATISTICAL ANALYSIS

Data was analyzed by paired sample t-test and the level of significance (0.05) was used to validate and confirm the efficacy of Unani formulation using IBM SPSS statistics (version 25) software. Sample size calculation software (version 2.1.31) was used to calculate the sample sizes.

## RESULTS

### Preparation of the formulation (Tablet Friability test)

The friability value of the *Picolin-E* tablets was 0.2%, there was no breakage or cracking of tablets during the experiment. Tablets loss was less than 1% of their mass during the friability test and hence was considered acceptable (Pharmacopeia, 2019).

### Pre-clinical studies

The antioxidant activity was measured by DPPH method, *Picolin-E* at 5mg/ml showed 56% while ascorbic acid (5 mg /ml) used as positive control demonstrated 82% anti-oxidant activity.

The anti-inflammatory activity of *Picolin-E* (15mg /kg) was carried out in rats by carrageenan induced rat paw edema and it demonstrated maximum 30.8% percent reduction in edema at 3<sup>rd</sup> hour that was maintained till the 4<sup>th</sup> hour of observation. While the standard drug diclofenac sodium group (10mg/kg) demonstrated maximum 36.5% percent reduction in edema at 2<sup>nd</sup> hour but it gradually declined to 26.5% at 3<sup>rd</sup> and 4<sup>th</sup> hour respectively (table 2).

Prior to clinical studies, acute toxicity of the hydroalcoholic extract Unani formulation was evaluated. There were no signs of any behavioral changes, undesirable effects, morbidity and mortality in female albino mice during 14 days of observation up till 5g/kg.

### Clinical studies

A total of 73 patients completed the treatment course, 39.7% of the patients were in the age group of 22-25 years. The effect of capsule *Picolin* before and after

**Table 1:** Distribution of the polycystic ovarian syndrome patients according to age

S. No.	Age group (Years)	Picolin (Capsule)	Picolin-E (Tablet)	Metformin (Tablet)	Total (n)
1	18-21	6 (24)	0 (0)	6 (24)	12 (16.4)
2	22-25	10 (40)	15 (65.2)	5 (20)	29 (39.7)
3	26-29	7 (28)	6 (26.1)	8 (32)	22 (30.1)
4	30-35	2 (8)	2 (8.7)	6 (24)	10 (13.7)
Total		25	23	25	73

The values represent the frequency of PCOS patients receiving either *Picolin* (n=25), *Picolin-E* (n=23) or metformin (n = 25) while the percentages of age distribution are within parentheses.

**Table 2:** Percent inhibition by *Picolin-E* in carrageenan-induced rat paw edema

S. No.	Sample	Time interval				
		30 min	1 h	2 h	3 h	4 h
1.	<i>Picolin-E</i>	-	7.6	24.7	30.8	30.8
2.	Diclofenac sodium	10	15.3	36.5	26.5	25.5

The values represent percent inhibition of edema in rat paw after treatment with either *Picolin-E* (15mg/kg) or Diclofenac sodium (10mg/kg) during different time intervals (hours, h) as compared to untreated control group (n=6).

**Table 3:** The effect of *Picolin* capsule, *Picolin-E* tablet and metformin tablet on hormonal levels of PCOS patients

Serum Parameters	<i>Picolin</i> (Capsule)		<i>Picolin-E</i> (Tablet)		Metformin (Tablet)	
	Before	After	Before	After	Before	After
Insulin ( $\mu$ Unit/mL)	14.8 $\pm$ 1.8	10.7* $\pm$ 1.7	16.7 $\pm$ 1.7	9.3* $\pm$ 0.1	17.4 $\pm$ 1.6	16.4 $\pm$ 1.4
Prolactin (ng/mL)	17.7 $\pm$ 0.9	8.5* $\pm$ 1.1	18.5 $\pm$ 1	10* $\pm$ 1.5	21.7 $\pm$ 2.8	21 $\pm$ 2.8
SHBG (nmol/L)	48 $\pm$ 6.3	55 $\pm$ 6.5	52 $\pm$ 6	55 $\pm$ 5.6	47.3 $\pm$ 3.8	47.6 $\pm$ 10.6
Total testosterone (ng/mL)	0.35 $\pm$ 0.05	0.5 $\pm$ 0.07	0.4 $\pm$ 0.01	0.8 $\pm$ 0.05	5.5 $\pm$ 17	5.4 $\pm$ 17
FSH (mIU/mL)	4.4 $\pm$ 0.5	4.5 $\pm$ 0.2	3 $\pm$ 0.4	2.5 $\pm$ 0.2	5 $\pm$ 0.4	5.1 $\pm$ 0.3
LH (mIU/mL)	8.9 $\pm$ 1.5	6.3 $\pm$ 1.1	5 $\pm$ 0.2	6.8 $\pm$ 1.1	10.2 $\pm$ 1.6	9.7 $\pm$ 1.5

*Picolin* (n = 25), *Picolin-E* (n = 23) or Metformin (n = 25), Duration of all treatments = 12 weeks, Sex hormone binding globulin (SHBG), Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Asterik\* indicates significant values Mean  $\pm$  S.E.M as compared to control while non-significant are without any sign.

**Table 4:** Effect of *Picolin* capsule, *Picolin-E* tablet and metformin tablet on menstrual flow in PCOS patients

Improvement response	n	<i>Picolin</i> (Capsule) (%)	<i>Picolin-E</i> (Tablet) (%)	Metformin (Tablet) (%)
Yes	61	84	61	54
No	12	16	38	46

Patients (%) showing either improvement (Yes) or no improvement (No) in menstrual flow after treatment with *Picolin*, *Picolin-E* and metformin, Total number of patients = 73

treatment on serum insulin (14.8 $\pm$ 1.8 vs 10.7 $\pm$ 1.7 $\mu$ IU/mL) and prolactin (17.7 $\pm$ 0.9 vs 8.5 $\pm$ 1.1ng/mL) levels were significant. Likewise, the tablet *Picolin-E* also demonstrated significant effect on serum insulin (16.7 $\pm$ 1.7 vs 9.3 $\pm$ 0.1 $\mu$ IU/mL) and prolactin (18.5 $\pm$ 1 vs 10 $\pm$ 1.5ng/mL) levels. On the contrary in metformin treated group these parameters were non-significant (table 3). Improvement was noticed in the menstrual flow of PCOS patients after treatment with *Picolin* (84%), *Picolin-E* (61%), and in metformin (54%) respectively (table 4).

The most frequent adverse effects were mild to moderate and gastrointestinal in nature. The frequent adverse effects observed included: a) acne in both *Picolin* (12%) and *Picolin-E* (17%) treated groups. However, in

metformin group: a) nausea (16%), b) bloating (8%) and c) fatigue (4%) were common.

Only two types of temperaments (*mizaj*) were observed in the study subjects i.e. phlegmatic (*Balghami*, 95%) and melancholic (*Saudawi*, 5%) respectively.

## DISCUSSION

In general, the line of treatment in Unani medicine is based on improvement of *mizaj* of the disease. The PCOS has cold and moist *mizaj* that disturbs the phlegmatic humor hence the risk of this disease increases in individuals having cold and moist *mizaj* (Majoosi, 1889). The *mizaj* of an individual is not static but under the



**Fig. 1:** Different steps of *Picolin* capsule and *Picolin-E* tablet preparation

influence of various factors such as, environment, diet, lifestyle and psychological factors. Basically, excessive coldness and moisture (*Balghami*) leads to PCOS and the aim of the Unani treatment is its adjustment towards equilibrium. In our study ~95% of the patients were *Balghami* which is in agreement with the earlier study while 20% were of *Damwi mizaj* (hot and moist) (Khan *et al.*, 2017). On the contrary we observed only 5% *Saudawi mizaj* (cold and dry) suggesting that PCOS may occur in females with other types of *mizaj* but it is predominant in *Balghami* individuals. This finding supports Razi's claim

that menstrual irregularity and obesity in women is apparent in *Balghami* subjects (Razi, 2001).

In the present study the Unani formulation used consisted of four medicinal plants namely: 1) *Cinnamomum verum*, 2) *Glycyrrhiza glabra*, 3) *Linum usitatissimum* and 4) *Vitex agnus castus*, all with hot and dry *mizaj* opposing the nature of the disease. Considering that PCOS is primarily a hormonal disorder therefore serum fasting insulin, prolactin, testosterone, SHBG, FSH and LH levels were determined before and after treatment.

It is well established that insulin resistance in PCOS leads to hyperinsulinemia (Rojas *et al.*, 2014). The Unani polyherbal formulation, *Picolin* and *Picolin-E* demonstrated 29% and 46.8% reduction, respectively in the serum fasting insulin levels of PCOS patients, however *Picolin-E* appears to be 1.5x more effective. The dose of cinnamon in *Picolin* used for 12 weeks was 600 mg/ day (200 mg/ 3 times a day) while for hydroethanolic extract *Picolin-E* it was 150mg/day (50mg/3 times a day), later being 4x lower in dosage but more effective probably due to the major water soluble active constituent procyanidin type - A polymers residing in it which upregulates glucose uptake, increases glycogen synthesis by activating glycogen synthase and enhances the insulin receptor sensitivity (Couturier *et al.*, 2011). Similar responses in PCOS patients were also observed consuming only cinnamon powder as 1.5 g/day (500 mg/ 3 times a day) for 12 weeks (Hajimonfarednejad *et al.*, 2018). Considering the dose of pure cinnamon used versus *Picolin-E* and *Picolin* were 10x and 2.5x lower, respectively. Another component of the Unani formulation, flaxseed has also been reported to lower serum insulin levels (Haidari *et al.*, 2020). This has been attributed to the presence of lignan (secoisolariciresinol diglucoside) which enhances the insulin signalling and upregulating the expression of insulin dependent glucose receptor (GLUT4) (Wang *et al.*, 2015).

Metformin, a bi-guanide reduces insulin secretion and hyperinsulinemia thereby improving insulin sensitivity and ovary function (Ou *et al.*, 2017). Metformin use at 1g (500mg/twice a day) caused only 5.8% reduction in serum insulin levels in PCOS patients. However, a considerable (23%) decline was observed after treatment with metformin 1.5g (500mg/3 times a day) for 12 weeks (Sharma *et al.*, 2019). This discrepancy in the magnitude of reduction is probably due to a smaller dose used.

In serum prolactin levels in PCOS patients a similar reduction (~44%) was evident after *Picolin* 900mg/day (300mg/ 3 times a day) and *Picolin-E* 225mg/day (75 mg/3 times a day) probably due to the presence of *Vitex agnus castus*. Likewise, the patients with hyperprolactinemia after treatment with formulation containing its extract (40mg /day) for 12 weeks also reduced (42%) prolactin levels (Awan, 2019). However, in our formulation *Picolin E* and *Picolin* the content of *Vitex agnus castus* was 15x and 1.8x higher. This difference between them may be related to the nature of other herbs or additives used in the former studies supporting the role of synergism or interaction with other herbs with opposing effects. Apparently, a diterpene residing in *Vitex agnus castus* stimulated the dopamine D2 receptor and hence attenuated the prolactin secretion (Nasri *et al.*, 2007) thereby improving the menstrual cycle regularity (Wang *et al.*, 2012). Metformin induced a non-significant reduction (3.2%) in the prolactin levels of our

PCOS patients which might be due to the lowest levels within the normal range (Krysiak *et al.*, 2016).

The high levels of testosterone in PCOS patients disturbs the process of ovulation leading to menstrual irregularities (Rojas *et al.*, 2014). In our study metformin and both Unani formulations were ineffective in lowering serum testosterone levels significantly despite the presence of flaxseeds as one of its components. This is more likely due to low base line testosterone levels in our subjects which is supported by the fact that in 20-40% women with hirsutism and polycystic ovaries serum androgens are not elevated (Richardson, 2003). Nevertheless, flaxseed (30g/day) used for a duration of 4 months caused a significant reduction (70-89%) in testosterone levels (Nowak *et al.*, 2007). Furthermore, licorice inhibits 17 $\beta$ -hydroxysteroid dehydrogenase thereby preventing the conversion of 17-OH-progesterone to androstenedione and testosterone and also inhibits 5 $\alpha$  reductase responsible for the conversion of the testosterone to its active form dihydrotestosterone (Armanini *et al.*, 2002) thereby attenuating menstrual irregularity, anovulation and hirsutism (Rojas *et al.*, 2014). Additionally, the former formulation also contains licorice possessing anti-androgenic activity (Nowak *et al.*, 2007; Helal *et al.*, 2019).

A sex hormone carrier protein SHBG reduces the amount of free circulating testosterone (Goldman *et al.*, 2017). A slight but non-significant rise in SHBG levels was induced by *Picolin* (7.5%), *Picolin-E* (1.8%) and metformin (2.1%). Likewise, flaxseed powder at a high dose i.e. 30g/day for 12 weeks in PCOS patients was also not effective in stimulating the SHBG levels (Haidari *et al.*, 2020) which might indicate flaxseed does not play any important role in improving SHBG levels.

Menstrual irregularity is one of the clinical manifestations of PCOS disease (Goodarzi *et al.*, 2011). High levels of insulin stimulate ovarian androgen production which ultimately inhibits ovulation leading to menstrual irregularity (Rojas *et al.*, 2014). In the PCOS patients *Picolin*, *Picolin-E* and metformin demonstrated 84%, 61% and 54% improvement in menstrual cycle and flow respectively. Likewise, improvement in menstrual cycle was observed by cinnamon (52%) at a dose of 3 g/day (1.5 g twice a day) and metformin (61%) 1g (500 mg twice a day) for 6 months (Khan and Begum, 2019). Cinnamon has hot *mizaj* and possess emmenagogue property and hence stimulates menstrual blood flow (Zare *et al.*, 2020). This correction of *mizaj* and induction of menstruation of PCOS patients is according to the principles of Unani treatment (Razi, 2001).

Furthermore, patients with dysmenorrhea showed reduction in pain after treatment with both *Picolin* and *Picolin-E* suggesting its substantial anti-inflammatory activity. The anti-inflammatory activity against

carrageenan induced rat paw edema at the 3<sup>rd</sup> hour of the experiment was induced by *Picolin-E* which was 1.1x greater than the standard drug diclofenac sodium. This is more likely due to cinnamaldehyde present in cinnamon which has anti-spasmodic activity and also due to eugenol that inhibits the biosynthesis of prostaglandins and hence reduces inflammation. (Bani *et al.*, 2014). Additionally, obesity and insulin resistance in PCOS has been associated with chronic low-grade inflammation (Bannigida *et al.*, 2020), emphasizing that anti-inflammatory activity of the Unani preparations are highly beneficial.

The PCOS disease also causes oxidative stress consequently lowering the serum anti-oxidants levels in the patients (Alipour *et al.*, 2019) thereby increasing the risk of cardiovascular disease, insulin resistance, hypertension and dyslipidemia (Wang *et al.*, 2019). *Picolin-E* demonstrated considerable antioxidant potential of 56% measured by DPPH method which was 26% lower than the standard ascorbic acid. Thus, PCOS patients can be recommended to include anti-oxidants for improving the symptoms and preventing other complications.

In all groups mild to moderate adverse effects were also observed which were mostly reversed within 4 weeks. The leading complaint in both Unani formulations *Picolin* (12%) and *Picolin-E* (17%) was acne which may be due to the excessive heat produced owing to the hot *mizaj* of the formulation used. In metformin group, nausea (16%), bloating (8%) and fatigue (4%) was common. While digestive system related adverse effects in 20% of the patients receiving metformin has been reported (McCreight *et al.*, 2016). Despite of minor undesirable effects of the preparations none of the participants discontinued the treatment. Additionally, a few patients in both the Unani formulations groups also experienced better digestion and improvement in bowel habits.

It has been demonstrated that PCOS patients consumed higher amounts of simple carbohydrates, polyunsaturated fatty acids and lower amounts of fiber and monounsaturated fatty acids. This unhealthy dietary pattern is associated in aggravating the clinical sign and symptoms of PCOS (Hameed *et al.*, 2017; Zubair, 2018). This further justifies that lifestyle modification cannot be ignored in the management of PCOS which was not addressed in our study and the patients were free to continue with daily life routine. Therefore, it is suggested that lifestyle modification in combination with our treatment would produce better outcome in PCOS patients.

## CONCLUSION

Unani formulation *Picolin* either in hydroethanolic extract or powdered form were effective in PCOS patients as

reflected by the reduction in serum insulin and prolactin levels. It also improved the menstrual flow comparable to metformin. Additionally, the anti-inflammatory, anti-oxidant properties and non-toxic nature favours Unani formulation with a promising future to be included in a drug development program. However, its mechanism of action along with detailed toxicological studies needs to be explored.

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