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Honey Bee Venom Modulates Hyperglycemia in Response to Hyperandrogenism in Polycystic Ovarian Syndrome-Induced Wistar Rats

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Abstract

Polycystic Ovarian Syndrome (PCOS) is inflammatory disease characterized by hyperandrogenemia, hyperthecosis and chronic anovulation. Honey bee venom (HBV) contains a variety of biologically active components having various pharmaceutical properties. This study was designed to detect the possibility of HBV application as an anti-inflammatory therapeutic agent. To induce PCOS, 2mg/100gr B.W Estradiol Valerate (EV) was subcutaneously injected to induce PCOS in mature Wistar rats then ovaries and serum from three groups of EV-induced PCOS, HBV-treatment and normal intact animals were collected for histological comparison and blood sugar test. As a result, a significant increase in ovarian weight was observed in experimental group rather than controls. Furthermore, in HBV-treated group a significant decrease was observed in ovary weight comparing with experimental group ($P<0.01$). The results obtained from Chemo Luminesance Immuno Assay (CLIA) declared that testosterone and Estradiol levels in experimental group significantly increased ($P<0.001$). These hormones were decreased in animals treated with HBV. Blood sugar level showed reduction in HBV-treated rats. Thickness of theca layer, number and diameter of cysts significantly decrease in HBV group comparing to PCOS group. Moreover, corpus luteum, as a sign of ovulation, was observed in HBV-treated ovaries. In conclusion our results suggest that beneficial effect of HBV against PCOS may be mediated by the inhibitory effect of HBV on TNF-α level.

Keywords: Polycystic ovarian syndrome, honey bee venom, blood sugar, theca layer

1. Introduction

Polycystic Ovarian Syndrome (PCOS) is an inflammatory disease characterized by hyper androgenemia, hyperthecosis, hyperglycemia and chronic anovulation(1-6). Honey bee venom (HBV) contains a variety of biologically active components like peptides (Melittin and Apamin), enzymes and biologically active amines (histamine, epinephrine. It has shown that HBV has analgesic, anti-cancer and anti-inflammatory activity. Melittin, the major active ingredient of BV, has been reported to induce apoptosis and to possess anti-proliferation effects (7-9). TNF-α is a key inflammatory stimulus which plays a main role in regulating normal activity of ovary in follicular growth and luteal stages. It's over expression in adipose tissue leads to obesity and

insulin-resistance in humans and rodents. This factor by stimulating mitotic activity in undifferentiated theca cells and increasing steroidogenic cells causes PCOS (10-13). Whereas the anti-inflammatory and anticancer effects of HBV were proved, we examined sugar levels in rats with PCOS before and after treatment via HBV. We also measured plasma testosterone and theca layer thickness, the commonly used index of PCOS (1). We hypothesized that HBV can modulate hyperglycemia in bee venom-treated rat with PCOS in response to androgenemia, compared with age-matched controls.

2. Materials and Methods

Experiments were performed on Female Wistar rats (170±20g). Before and during the experiment they were housed in special cages with a standard space and under controlled cycle of light and darkness (lights on from 06:00 to 20:00), humidity 55±15% and temperature range of 20-24˚c and free access to water and commercial food (Behparvar Com., Iran). Induction of PCOS was administered using 1mg/100gr B.W intramuscular injection of EV. After verifying the induction of PCOS, experimental group was divided into two groups: PCOS group and PCOS+BV group. PCOS+BV received 0.5 mg/kg BV sc for 14 days, continuously (7). PCOS group in this period of time received physiological saline solution. At around 09:00 am, trunk blood was collected and the serums were separated using 4000 RPM centrifuge for 10 min. The ovaries were separated from the twisted oviduct tubes and were placed in bouin fixative for histological analysis; fixed samples were kept in alcohol solutions of 20 to 100% for a period of 45 min for dehydration and afterwards in alcohol/xylen (50:50) and xylen (3 times) for clearing and blocked in paraffin. The samples were sliced in 7 micron thickness using a microtome and the sections were placed on slides previously coated with gelatin and then stained with hematoxylin-eosin for histological observation. Serological analysis was performed to measure sugar level and hormone alterations. Testosterone and estradiol detected by Chemo Luminesance Immuno Assay (CLIA). In order to detect suger, glucose kit (GOD_PAP 90014) was used. The one-way ANOVA and INSTAT software were used to determine the statistical significance of differences between the values for the experimental and control groups. Data are expressed as means ± standard errors (S.E.M) and the results are taken from at least three independent experiments performed in triplicate. P-values of 0.05 or less were considered statistically significant.

3. Results

The ovaries were also precisely weighted and a significant increase was observed in experimental group rather than controls. Furthermore, in bee venom-treated group a significant decrease was observed in ovary weight comparing with experimental group ($P<0.01$). The results obtained from CLIA declared that testosterone and Estradiol levels in experimental group significantly increased ($P<0.001$). These hormones were decreased in animals treated with bee venom, and comparing with animals in control group they were regulated. Reduction observed in testosterone and Estradiol levels was significant with $P<0.05$. These data including raise in androgens showed that induce of syndrome was absolutely successful. What more is bee venom managed to reduce Estradiol and testosterone.(Table1) sugar level fundamentally adjusts its production, which in this study, PCOS induction by means of Estradiol volerate led to a significant raise in

sugar level (P<0.01), and its reduction in rats treated with honey bee venom was outstanding (P<0.05). (Diagram1).

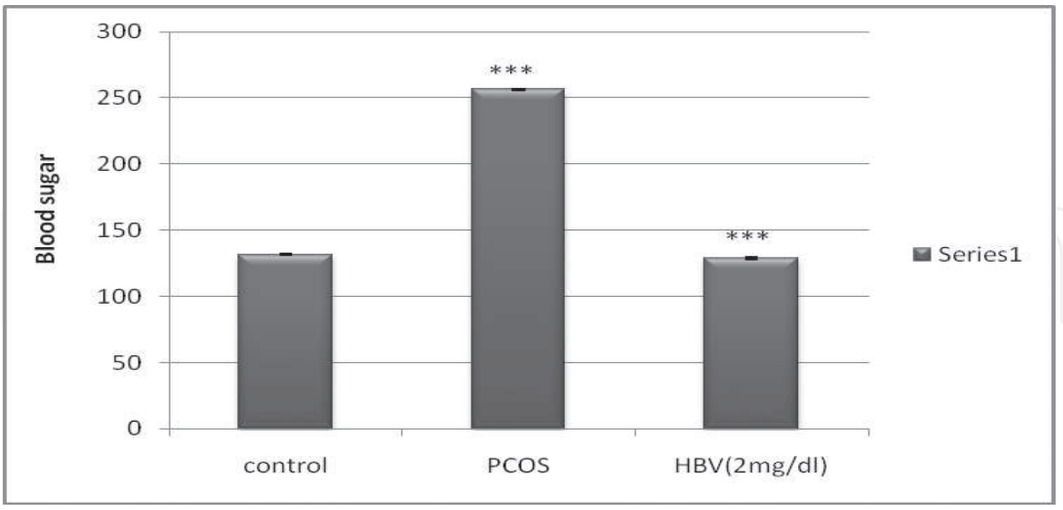

Diagram 1. Different sugar level in control and polycystic rats (n=10). In polycystic group, a significant increase was observed in sugar level. ***P< 0.001, PCOS vs. control, HBV vs. PCOS group.

	Cont	SD	SEM	PCOS	SD	SEM	PCOS+BV	SD	SEM	con vs pcos	con vs pc+bv	pcos vs pc+bv
Estradiol (pg/ml)	14.5	1.958	0.6191	54.778	8.599	2.866	21.889	4.485	1.495	*** P<0.001	* P<0.05	*** P<0.001
Testosteron (ng/dl)	62.5	9.925	3.138	345.9	112.99	35.73	115.9	39.145	12.379	*** P<0.001	ns P>0.05	*** P<0.001
Ovarian weight (mg)	13	3.232	1.022	20.5	4.577	1.447	15	4.714	1.491	** P<0.01	ns P>0.05	* P<0.05
Theca layer -late antral follicles (micrometer)	99.8	11.144	3.524	157.2	44.183	13.972	110.7	23.281	7.362	*** P<0.001	ns P>0.05	** P<0.01

Table 1. Bee venom treatment effects in polycystic ovarian syndrome (PCOS). Baseline parameters of polycystic ovarian syndrome (PCOS) rats (n=10) and control (n=10) and bee venom –treated rats (n=10).

In order to determine follicular development, follicles were classified based on morphology and diameter into 6 groups consisting of: primordial, primary and preantral (<600µm), antral (600-1000µm), cystic follicles, and corpus luteum. Decrease in the number of primary follicles, antral follicles and corpus luteums was significant with P<0.001, in primordial follicles with P<0.01, and in preantral follicles with P<0.05. In PCOS ovaries, some large cystic follicles with thick theca layer were observed. In this group, no corpus luteum as a sign of ovulation was seen.

In rats treated with honey bee venom, the number of primordial and preantral follicles and corpus luteums increased, whereas, the number of cysts and thickness of theca layer in antral follicles decreased, which these changes were significant in comparison with sham group. Also, some corpus luteums were observed in this group which was considered as a sign of relative improvement in PCOS ovaries. (Diagram 2 and 3).

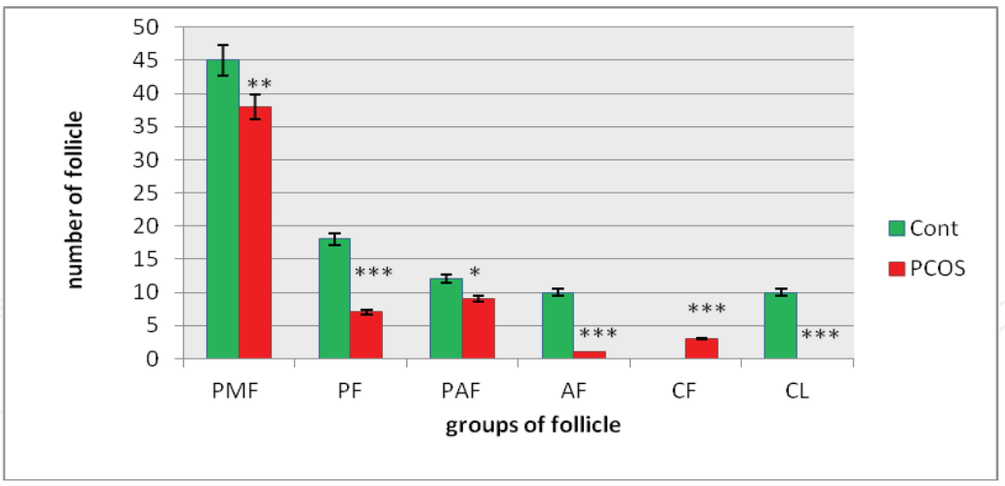

Diagram 2. Different follicular groups in control and polycystic ovaries (n=10). In ovaries of polycystic group, a significant increase and decrease was observed in number of cysts and number of corpus luteums respectively. ***P< 0.001, **P< 0.01, *P< 0.05. (PMF, Pre Mordial Follicle; PF, Primary Follicle; PAF, Preantral Follicle, AF, Antral Follicle; CF, Cystic Follicle; CL, Corpus Luteum.)

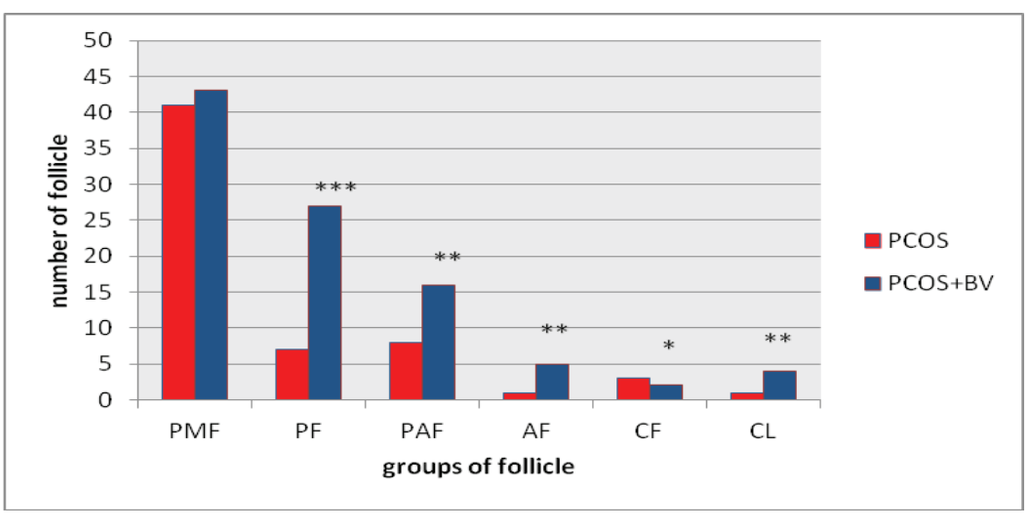

Diagram 3. Different follicular types in ovaries of polycystic and bee-venom treated polycystic group. In polycystic ovaries treated with honey bee venom (n=10), a significant increase was observed in all follicular clusters (except primordial follicles) rather than polycystic group. The primordial follicles were not significantly increased. Moreover, a significant decrease was seen in the number of ovarian cysts. ***P<0.001, **P< 0.01. (PMF, Pre Mordial Follicle; PF, Primary Follicle; PAF, Preantral Follicle, AF, Antral Follicle; CF, Cystic Follicle; CL, Corpus Luteum.)

4. Summary and conclusion

Insulin sensitivity and hyperglycemia is directly related to androgen levels (14). These findings suggest that hyperandrogenism play a role in the development of insulin resistance and hyperandrogenism in PCOS. It is considered metformin as a treatment for PCOS which has been shown to inhibit the NF-κB activation and also reduce sugar level in PCOS woman (15). Then we can also name honey bee venom as a similar factor decreasing sugar level. Histological changes

observed in ovary after bee venom treatment, can also be considered as a confirmation for this syndrome progress. Our results confirm that Bee venom caused a decrease in follicular theca layer in PCOS rats, which is actually because of increased lipolysis and decreased hypertrophy of this layer. Due to this decrease, the androgens and steroids produced by this layer also decrease and consequently the total levels of serumic estrogen and androgens reduce by honey bee venom. In this regard we have demonstrated that bee venom injection produces a significant anti-hyperglycemia effect in PCOS Wistar rats.

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