Effects of naloxone and yohimbine in polycystic ovary syndrome: a rabbit model study

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Background/aim: To assess the therapeutic effects of naloxone and yohimbine on polycystic ovary syndrome (PCOS) in a rabbit model in terms of body weight and endocrinological parameters (luteinizing hormone, insulin, and estradiol).

Materials and methods: A total of 50 adult, reproductively mature female rabbits (*Oryctolagus cuniculus*) were divided into five groups (n = 10/group). In the control group PCOS was not induced (negative control group), whereas in the remaining four groups (n = 40) PCOS was induced with a single i.m. injection of testosterone daily and were designated as follows: positive control, naloxone-treated (NalT), yohimbine-treated (YohT), and naloxone+yohimbine-treated (NalYT) groups.

Results: A steadily ascending trend was noted in all of the studied parameters in the PCOS-induced group as compared to the negative control group. All the parameters showed a descending trend in the NalT group as compared to the positive control. Regarding the YohT and NalYT groups, all parameters showed a descending trend as compared to the positive control group except for estradiol.

Conclusion: Naloxone therapy either alone or combined with yohimbine improves a wide range of the clinical manifestations of PCOS. Furthermore, we suggest this therapy as an alternative to the conventional therapy with insulin-lowering agents in vogue.

Key words: Polycystic ovary syndrome, naloxone, yohimbine

1. Introduction

Although polycystic ovary syndrome (PCOS) is heterogeneous in nature and presents a wide variability in its clinical manifestations, its primal indicators include ovulatory dysfunction and hypergonadism (1). Its correlation with hyperactivity of the sympathetic nervous system, stress, and alteration in β-endorphins has been extensively reported (2,3). Deregulation of the opioid system influences the pancreatic β-cell function and ultimately leads to obesity and PCOS (4). Furthermore, an intricate relation between endogenous opioids, insulin, and GnRH output has previously been elaborated (5,6).

Women of reproductive age belonging to all races and nationalities have been found equally prone to PCOS (7). Its presentation may, however, be variable among different races and in people belonging to different geographical regions (8). Wide variance has been reported regarding its prevalence among populations and has been attributed to the effect of ethnic origin, race, and environmental factors on phenotype (9,10). A 20.7% prevalence of PCOS in the female population of Pakistan has been reported (11). However, a more recent work has reported the prevalence to be 81% and 44% in women with and without hirsutism, respectively (12).

Owing to its multietiological patterns, the treatment regimens for PCOS are multifaceted and depend upon its patternization (8). Insulin-lowering therapies have been used as one of the regimens (13). However, the latest trends include the use of drugs inhibiting the sympathetic nervous and opioid system. The present study is the first of its kind reported from Pakistan regarding the therapeutic aspects of PCOS using naloxone and yohimbine in terms of body weight and certain endocrinological parameters in a rabbit model.

2. Materials and methods

2.1. Study animals and management

A total of 50 adult, reproductively mature female rabbits (*Oryctolagus cuniculus*) ranging in age from 10 to 12 months were purchased from a local market in Lahore, Pakistan. They were acclimatized to their housing and feeding regimen for 2 weeks prior to the start of trial at the animal house of Lahore College for Women University, Lahore, Pakistan. An appropriate diet consisting of green fodder given three times a day; vegetables, fruits, and dry feed (grains such as wheat, corn and oat) twice a week; and free access to water ad libitum was maintained.

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2.2. Experimental design

After acclimatization, all the rabbits were randomly divided into five groups (n = 10/group). In the control group PCOS was not induced and it was denoted as the negative control group. The remaining animals (n = 40), however, were given a single i.m. injection of testosterone (Testosterone Depot, Schering, Berlin, Germany) daily for 4 weeks at the dosage rate of 4 mg/kg for the induction of polycystic ovaries (14,15). After inducing PCOS, they were assigned to four groups (n = 10/group) as described below.

The first group was given no treatment and was denoted as the positive control group.

The second group (NalT) was administered 0.1 mg/kg of naloxone i.m. (naloxone hydrochloride inj., USP 0.4 mg/mL, Hospira Inc., Maidenhead, UK) for the inhibition of the opioid system (16).

The third group (YohT) was given 1 mg/kg of yohimbine i.m. (yohimbine hydrochloride inj., Lloyd Inc., Shenandoah, IA, USA) for the inhibition of the sympathetic nervous system (16).

The fourth group (NalYT) was administered both 0.1 mg/kg naloxone and 1 mg/kg yohimbine i.m. for the inhibition of both the opioid and sympathetic nervous systems (16).

The treatments were carried out on a daily basis for a total of 5 weeks during which weekly monitoring was maintained for the assessment of body weight and endocrinological parameters.

2.3. Sample collection and analysis

Blood samples (4 mL) were collected aseptically from the marginal ear vein of each animal, on a weekly basis, under a proper restraining protocol. They were transferred into vacutainers containing thixotropic gel separator for serum separation. Serum was harvested and analyzed for luteinizing hormone (LH), insulin, and estradiol through commercially available enzyme linked immunosorbent assay (ELISA) kits as described in Table 1. Body weight was measured prior to each sample/blood collection.

2.4. Statistical analysis

Statistical analysis was conducted with the Statistical Package for the Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± SE. The statistical difference between the positive and negative control groups was assessed by t-test whereas that between various treatments was tested through repeated measures analysis of variance, followed by Bonferroni post-hoc test.

3. Results

The comparative means (±SE) of body weight and certain endocrinological parameters between the negative and positive control (PCOS-induced) groups are presented in Table 2. A steadily ascending trend was observed in all of the studied parameters for the PCOS-induced group across the study weeks. Statistical significance was, however, achieved in varying weeks and at varying level. Body weight was significantly higher in weeks 3, 4, and 5 ($P < 0.05$); LH in weeks 2 and 3 ($P < 0.05$), and 4 and 5 ($P < 0.01$); estradiol in weeks 1, 2, and 3 ($P < 0.05$), and 4 and 5 ($P < 0.01$); and insulin in weeks 1, 2, 3, 4, and 5 ($P < 0.01$).

The comparative means (±SE) of body weight and certain endocrinological parameters between the PCOS-induced (positive control) group and the various treatment groups are presented in Figure 1. All the parameters showed a descending trend in the NalT group as compared to the positive control group. Statistical significance was, however, achieved in varying weeks and at varying level. Body weight was significantly lower in weeks 4 and 5 ($P < 0.05$); LH in weeks 3 and 4 ($P < 0.05$), and 5 ($P < 0.01$); estradiol in weeks 2 and 3 ($P < 0.05$), and 4 and 5 ($P < 0.01$); and insulin in weeks 4 ($P < 0.05$) and 5 ($P < 0.01$).

Regarding the YohT group, all parameters showed a descending trend as compared to the positive control group except for estradiol. Body weight was significantly lower in weeks 4 and 5 ($P < 0.05$); LH in weeks 1 ($P < 0.05$) and 2, 3, 4, and 5 ($P < 0.01$); and insulin in weeks 1, 2, 3, 4, and 5 ($P < 0.01$). Estradiol, in contrast, revealed an

Table 1. Detail of commercial kits used for assessment of various hormones of the study.

Attribute	Make and batch no.	Precision %		Sensitivity
		Within assay	Between assay	
Luteinizing hormone	LH ELISA Kit, FR E-2600, Labor Diagnostika Nord GMBH & Co. KG. Germany	4.5, 2.7, 2.9	5.1, 8.1, 9.2	0.2 IU/L
Estradiol	Estradiol ELISA Kit, BC1111, Biocheck, Inc. Foster City CA, USA.	24.1, 10.3, 4.1, 4.9	26.7,10.3, 6.4, 6.6	10 pg/mL
Insulin	Insulin ELISA Kit, YK060, HOLZEL Diagnostika, Hansaring, Deutschland (Germany).	2.4, 3.0, 4.2	5.8, 5.0, 3.8	0.5 U/mL

Table 2. Body weight and certain endocrinological parameters between negative and positive control (PCOS-induced) groups. Values are presented as mean ± SE. * P < 0.05; **P < 0.01.

Parameters	Weeks					
	Groups	Week 1	Week 2	Week 3	Week 4	Week 5
Body weight (kg)	Negative control	1.05 ± 0.02	0.96 ± 0.04	0.98 ± 0.03	0.98 ± 0.03	0.99 ± 0.01
	Positive control	1.05 ± 0.05	1.17 ± 0.04	1.25 ± 0.05*	1.29 ± 0.05*	1.29 ± 0.07*
Luteinizing hormone (IU/L)	Negative control	2.03 ± 0.02	2.10 ± 0.03	1.88 ± 0.09	1.98 ± 0.01	1.84 ± 0.03
	Positive control	3.76 ± 0.24	4.31 ± 0.13*	4.68 ± 0.09*	5.25 ± 0.16**	5.24 ± 0.14**
Estradiol (pg/mL)	Negative control	5.08 ± 0.04	5.08 ± 0.04	4.38 ± 0.19	4.33 ± 0.15	4.35 ± 0.12
	Positive control	13.80 ± 0.18*	15.10 ± 0.10*	16.10 ± 0.14*	17.60 ± 0.17**	17.7 ± 0.17**
Insulin (U/mL)	Negative control	1.42 ± 0.36	1.15 ± 0.35	0.92 ± 0.23	0.93 ± 0.22	0.97 ± 0.22
	Positive control	8.00 ± 1.77**	8.59 ± 1.85**	9.36 ± 1.76**	10.21 ± 1.79**	10.22 ± 1.02**

ascending trend as compared to that in the positive control group, being significantly higher in weeks 2 (P < 0.05), and 3, 4, and 5 (P < 0.01) (Figure 1).

For the NalYT group, the mean results in comparison to the positive control group were the same as those for the YohT group. A descending trend was noted for all parameters except estradiol, which was found to be higher in weeks 2 (P < 0.01) and 3, 4, and 5 (P < 0.05) (Figure 1).

4. Discussion
A steadily ascending trend was seen for all of the studied parameters (body weight, LH, insulin, and estradiol)
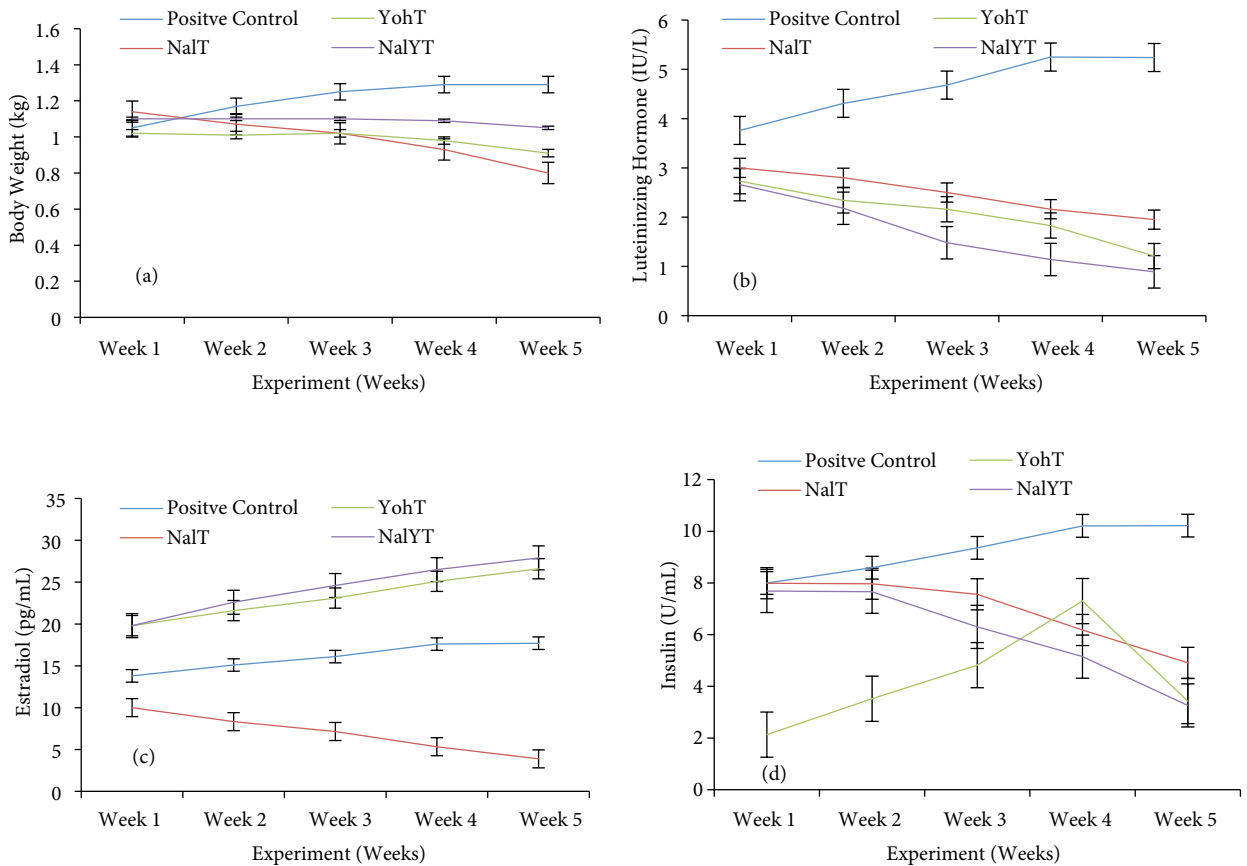

Figure 1. Body weight (a), luteinizing hormone (b), estradiol (c), and insulin (d) levels between the negative and positive (PCOS-induced) groups.

for the PCOS-induced group across the study weeks. It has been established that PCOS is characterized by obesity and/or overweight as compared to non-PCOS women (17), elevated LH (18), insulin (19), and estradiol (20) hormones. In present study, PCOS was induced in the animals by injecting testosterone (4 mg/kg) for 4 consecutive weeks. This leads to increased levels of circulatory androgens (testosterone), which are then converted into estrogens by aromatase activity (21). However, in PCOS animals, increased amplitude and frequency of LH pulse can be attributed to rapid GnRH stimulation through the positive feedback mechanism of the circulatory estrogens on adenohypophysis (18). This increased LH further produces androstenedione from stromal and thecal cells of the ovary and finally it is again converted to estrone, exerting a tonic effect on LH production. Regarding the elevated insulin concentration in the positive control group, it can be attributed to short-term testosterone treatment causing alterations in the expression of lipolytic signaling proteins (22) of bodily adipose tissue in relation to lipolytic activity and insulin resistance (23) in the PCOS group. Furthermore, insulin resistance and hyperinsulinemic conditions through their individual or combined effect contributed towards increased body weight in the PCOS group (24).

Naloxone is an opioid receptor antagonist and causes blockage of hypothalamic opioid receptors and reduces the opioid tone (5). It is used for the inhibition of the opioid system, for overdose reversal, and as a therapeutic agent (19). In the present study, body weight, LH, estrogen, and insulin showed a descending trend in the NalT group as compared to the positive control group. These results are consistent with various studies on PCOS that have elaborated that the opioid antagonist naltrexone has a positive effect on normalization of body weight (20,25), LH (19), estrogen (19), and insulin (26) in the PCOS group. Naloxone can be suggested to impart its effect on body weight by decreasing opioid peptide β endorphin level (27,28) and regulating food intake through its effect on the appetite center (25). However, the decline in LH and estrogen level can be attributed to inhibition of GnRH by naloxone in hyperinsulinemic PCOS women (29). Similarly, the decrease in insulin level can be due to its action on insulin secretion by reducing it up to 30% without causing change in the composition of β cell secretion (20,30). Consequently, all these parameters were changed in PCO-induced animals and were observed to be normal after treatment with naloxone.

Yohimbine is an antagonist of the sympathetic nervous system and decreases sympathetic nerve activity and increases plasma norepinephrine level without increasing sympathetic nerve activity (5,31). Sympathetic and hypothalamic–pituitary–adrenal axis (HPA) activity is increased in PCOS subjects (32). Regarding the YohT group, all parameters showed a descending trend as compared to the positive control group except estradiol. A decline has been reported for body weight of the PCOS group after treatment with yohimbine earlier (33). Similarly, a decline in LH concentration after yohimbine therapy in the PCOS group and normalization of ovulatory cycles by increased concentration of estrogen in PCOS rats as compared to the control group has also been reported (31). These alterations in endocrinological parameters and body weight can be due to yohimbine-induced dysregulation in PCOS (34). However, alterations in ovulatory cycles as an increased concentration of estrogen in the PCOS group as compared to the control group are contrary to previous reports.

For the NalYT group, the mean results in comparison to the positive control group were the same as those for the YohT group. A descending trend was noted for all parameters except estradiol. It has been established that the opioid and sympathetic nervous systems interact and cause dysregulation in PCOS (34). Hyperinsulinemia, hyperandrogenism, insulin resistance, cardiovascular disorders, and obesity have been attributed to hyperactivity of the sympathetic nervous system (18,35). Therefore, for effective control of this increased activity of the opioid and sympathetic nervous systems in the PCOS group, naloxone and yohimbine were administered simultaneously to experimental animals in the present study. The descending trend in studied parameters (body weight, LH, and insulin) except for estradiol in the PCOS-induced animals was consistent with recent work (31,36). However, contrary to previous studies, the interaction of the opioid and sympathetic nervous systems results in increased concentration of estradiol in experimental animals as compared to the positive control group. This ascending trend in estrogen could be attributed to an adjunct interaction of naloxone- and yohimbine-induced dysregulation in PCOS.

In a nutshell, naloxone therapy either alone or combined with yohimbine improves a wide range of clinical manifestations of PCOS. We suggest this therapy as an alternative to the conventional therapy of insulin-lowering agents in vogue. Randomized controlled trials need to be carried out in order to confirm these findings. Furthermore, possible teratogenicity may also be examined.

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