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Hormonal and Immunological responses to Coleus forskohlii treatment in Female Rats with Experimentally Polycystic Ovaries Syndrome

Hoda M.L. Abd-Allah[1*], Mona M. AbdAlhady[2], Magda N.A. Abdelall[1], Sahar N. Mohamedy[1], Waheeb D.M. Alharbi[3] and Adil O. Bahathiq[3]

[1]Animal Health Research Institute, Zagazig Laboratory, Egypt

[2]Physiology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

[3]Physiology Department, Faculty of Medicine, Umm al Qura University, Makkah, KSA

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Abstract

Polycystic ovaries syndrome (PCOS) is one of the most prevalent causes of reproductive failure. Its pathogenesis may be hormonal and immune disorder with hyperactivity of the sympathetic nervous system. The present study was conducted to demonstrate the effect of coleus forskohlii roots extract (CFE) in modulating the levels of some hormones and immune parameters of rats with androgen induced PCOS. Fifty immature female albino rats were divided into control group and androgen treated group that injected subcutaneously with 60 mg/kg BW/day androgen (Cidotestone) for 35 days to induce PCOS. Each group was subdivided into two subgroups. The first two subgroups received saline and 25 mg/kg BW of CFE, respectively while the other two subgroups received the same doses of Cidotestone and Cidotestone + CFE, respectively for 3 weeks. Blood samples were collected at the end of the experiment to estimate the levels of corticosterone, beta endorphin, total leucocytic count (TLC), cluster of differentiation 4 (CD^+4), cluster of differentiation 8 (CD^+8), interleukin 2 (IL-2), interleukin 3 (IL-3), interleukin 4 (IL-4), immunoglobulin G (IgG) and immunoglobulin A (IgA). The results revealed a significant decrease in the concentrations of β endorphin, IL-2, TLC, CD^+4, CD^+8 and IgG, while corticosterone and IL-4 showed a significant increase in the androgen induced PCOS group. Rats treated with Cidotestone + CFE displayed normal values of all tested parameters except corticosterone, suggesting role of CFE in mediating both humoral and cellular immunity.

Keywords: Coleus forskohlii, Rats, Polycystic Ovaries Syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrinological disorder characterized by hyperandrogenism and ovulatory dysfunction [1] and its etiology remains obscure. PCOS assumed to be due to neuroendocrine defect leading to an exaggerated LH pulse frequency and amplitude, a deficiency in insulin action leading to hyperinsulinemia or ovarian changes in FSH response [2]. Interestingly there is a close relationship between the endocrine system, the sympathetic nervous system and the immune system [3]. Basic symptoms of PCOS such as anovulation and follicular cysts were produced in female mice by injection of estrogen, testosterone or cortisone during immune adaptive period resulting in interference with the final stage of thymus gland development that alters the evolution of self versus non-self recognition [4,5]. Additionally, Öner and Ozan [6] suggested that steroids forestall the production of regulatory T cells.

Rats with estradiol valerate induced PCOS have reduced hypothalamic β-endorphin concentration and an increase of μ–opioid binding reflecting a chronic up–regulation of the receptor in response to compromised β-endorphin input [7]. Leukocytes can express opioid receptors [8] and under certain circumstances also synthesize and release β-endorphin themselves providing the molecular mechanisms for communication with the neuroendocrine system [9]. It is documented that women with PCOS have decreased frequencies of circulating CD^+8T cells and natural killer (NK) cells [10], altered cytokine responses and an increased number of activated T cells in follicular fluid [11,12]. The cyclic adenosine monophosphate (cAMP) can differentially regulate multiple cell processes and act as a positive regulator of a number of

***Corresponding author email: (hoda72001@yahoo.com), Animal Health Research Institute, Zagazig Laboratory, Egypt.**

immune function genes [13], through different pathways; including cyclic nucleotide gated ion channels, cAMP-activated protein kinases, or exchange proteins directly activated by cAMP [14,15]. Since forskolin is one of cAMP generator and represents as an active constituent of coleus forskohlii plant [16]. Its role as aromatase-agonist-like drug that can directly induce follicular development and shorten the treatment course of PCOS women, irrespective of the hyperandrogen state [17]. Hence, the present study was conducted to determine if rats with androgen- induced PCOS have alterated carticosterone, β-endrophine and immune response. The possible role of coleus forskorskohlii roots extract in alleviation these effects were also studied.

Material and methods

Animals and experimental design

Fifty pre-pubertal female albino rats (23 days old) were obtained from laboratory animal unit Faculty of Veterinary Medicine, Zagazig University and kept at a temperature of 25±2°C with a 12 light: 12 dark cycle. All animals were freely accessed to pelleted food and water and kept for one week before the beginning of the experiment. Animals were divided into two equal groups: the first group was injected with saline subcutaneously (S.C) and the second group was injected with cidotestone (androgen synthetic steroid, Chemical Industries Development Company, Giza, A.R.E) 60 mg/kg BW/day for 35 days for inducing PCOS [18] that was verified by vaginal smear analysis and histopathological examination. After verifying the induction of PCOS, the first group was subdivided into two equal subgroups, the first subgroup was left as control group (saline S.C and per os (PO)) and the second subgroup was given CFE, 25mg/kg BW PO (Ethanolic extract of coleus forskohlii roots that prepared according to Farias *et al.* [19] and saline S.C. The second main group was subdivided into two equal subgroups, the first subgroup injected with cidotestone 60 mg/kg BW/day S.C + saline PO and the second subgroup was injected with cidotestone + CFE treatment group (with the same previous doses of cidotestone and CFE). All treatments were given daily for 21 consecutive days. Rats were fasted for 6 hours and then ten rats from each group were sacrificed and blood was collected in ice chilled heparinized tubes for hormones and immune analyses. Blood samples were centrifuged at 3000 rpm for 15min and plasma was separated, frozen and stored at 20°C until assay.

Biochemical analysis

Hormonal and some immune parameters were estimated by enzyme–linked immune-sorbent assay (ELISA) kits to quantify the plasma beta–endorphin (ELISA kit, cat. No. MBS 704725), coticosterone (ELISA kit, cat No. MBS 705109), cluster of differentiation 4 (CD^+4, ELISA kit, cat. No MBS 263383), cluster of differentiation 8 (CD^+ 8, ELISA kit, cat. No. MBS 2503027), interleukin 2 (ELISA kit, cat. No. MBS 723159), interleukin 3 (ELISA kit, cat. No MBS 730102), interleukin 4 (ELISA kit, cat. No MBS 175989), immunoglobulin A (ELLSA kit, cat. No. MBS2500774) and immunoglobulin G (ELISA kit, cat. No. MBS 261432). Blood was used to determine of leucocytic count (TLC) using a hematological analyzer (coulter Micro Diff II Coulter Electronics Ltd, USA).

Statistical analysis

All statistics for the present data were determined using ANOVA (F-Test) followed by LSD (Least Significant Difference) for comparative of means using SPSS version 14 [20].

Table 1: Effect of coleus forskohlii root extract (CFE) on corticosterone and beta endorphin in rats with androgen induced polycystic ovaries

Parameters	Treatments			
	Control	Androgen	Androgen+CFE[1]	CFE[1]
Corticosterone(ng/ml)	0.63±0.022^d	1.01±0.047^c	1.37±0.099^b	1.99±0.062^a
Endrophine (pg/ml)	68.79±2.85^b	54.93±1.57^c	61.29±1.99^c	89.79±2.85^a

Data represented as mean ± SE (standard error) and the different letter in the same row were statistically significant at ($p \leq 0.05$) using LSD (least significant difference). [1]CFE= Coleus forskohlii roots extract.

Results

The levels of corticosterone hormone and beta endorphin in androgen induced PCOS and after treatment with CFE were presented in Table 1. It was clear that corticosterone levels increased significantly both with androgen induced PCOS and after treatment with CFE in comparison with the control group. Whereas the levels of beta endorphin exhibited a significant decrease in androgen induced PCOS group compared with the control group. In contrast, their concentrations were elevated in androgen administrations with CFE or CFE alone when compared with the control group. The impact of androgen induced PCOS on total leucocytic count and the recurrences of CD4 and CD8T cells were shown in Table 2. All these parameters were significantly lower in rats with PCOS group when compared with the control group. However, CFE treatment could restore these parameters to their control values.

Table 3 revealed a significant decrease in the concentration of IL2 with a significant increase of IL4 in androgen induced PCOS group, while IL3 concentration did not differ in this group compared with the control group. Treatment with CFE abolished alteration in cytokines responses. The results revealed a significant decrease in the concentration of IgG in the hyperandrogenized group, while IgA concentration displayed no significant response within this group compared to the control. Treatment with CFE evoked a significant elevation in IgG concentrations.

Table 2: Effect of coleus forskohlii root extract (CFE) on total leucocytic count, CD+4 and CD+8 in rats with androgen induced polycystic ovaries

Parameters	Treatments			
	Control	Androgen	Androgen+CFE[1]	CFE[1]
[2]TLC X 10^3	8.75±0.52[b]	6.34±0.70[c]	7.89±0.60[bc]	10.76±0.25[a]
[3]CD4 (ng/mL)	0.51±0.022[c]	0.32±0.011[d]	0.78±0.043[b]	1.02±0.50[a]
[4]CD8(pg/mL)	18.7±0.54[b]	13.10±0.38[c]	25.78±1.39[a]	28.10±0.62[a]

Data represented as mean ± SE (standard error) and the different letter in the same row were statistically significant at ($p \leq 0.05$) using LSD (least significant difference).[1]CFE= Coleus forskohlii roots extract. [2]TLC= total leucocytic count. [3]CD4=cluster of differentiation 4. [4]CD8= cluster of differentiation 8.

Discussion

There is a possible link between ovarian functionality and immune response during cytogenesis induced by steroid hormones. The present data revealed a significant increase in the levels of corticosterone in both androgen induced PCOS and androgen+ CFE groups, while there was a significant decrease in the plasma level of β endorphin and recurrences of circulating CD^+4 and CD^+8T cells in the hyperandrogenized group when compared with the control group. Coleus forskohlii extract treatment elevated β endorphin and the T lymphocyte activity in hyperandrogenized rats to simulate the control values.

Glucocorticoid prereceptor metabolism enhanced in 5α-dihydroxytestosterone treated rats (a model of PCOS) that assessed by elevated intracellular corticosterone [21]. On the other hand, Keefe *et al*. [22] showed no differences in serum concentration of the adrenal steroids dihydroepiandrosterdione, cortisol, corticosterone and their 11–deoxy precursors in woman with PCOS suggested an increase in their production and clearance [23]. In the present study, elevated levels of corticosterone in hyperandrogenized rats treated with CFE may be due to forskolin-induced nuclear accumulation of the recognized corticotropin–releasing hormones transcriptional regulators [24] and enhanced the normal glucocorticoid receptor function producing adrenal glucocorticoid that is essential for homeostasis and survival during severe stress situation [24,25]. In the current study, β endorphin concentration was decreased in rats with androgen induced PCOS. Similar results were obtained by Lobo *et al*. [26] in human with PCOS and in rats with estradiol valerate induced PCOS [27], indicating upset β endorphin production with this disease, since β endorphin aroused a tonic inhibitory control of gonadotrophin hormones

pulse generator [28], leading to the suppression of plasma LH pattern that is characterized the steroid–induced PCOS model [7]. The cAMP is considered an important second messenger system in regulation of hormone secretion and proopiomelanocortin [29] as well as having a critical role in the differentiation of β endorphin neurons [30]. This may explain the normalization of β endorphin concentration after CFE (cAMP stimulating agent) administration.

Table 3: Effect of coleus forskohlii root extract (CFE) on interleukins and immunoglobulins in rats with androgen induced polycystic ovaries

Parameters	Treatments			
	Control	Androgen	Androgen+CFE[1]	CFE[1]
[2]IL2 (pg/mL)	211.8±6.92[a]	152.69±1.45[c]	195.4±1.63[b]	212±4.84[a]
[3]IL3 (pg/mL)	237.6±4.75	240±4.93	236±4.16	237.4±4.97
[4]IL4 (pg/mL)	137.8±1.68[b]	143.61±1.18[a]	134.77±1.49[b]	114±0.92[c]
IgG (ng/mL)	3.81±0.12[b]	2.71±0.180[c]	5.16±0.024[a]	5.44±0.133[a]
IgA (ng/mL)	41.38±0.921	42.70±1.53	38.28±1.41	38.02±0.904

Data represented as mean ± SE (standard error) and the different letter in the same row were statistically significant at ($p \leq 0.05$) using LSD (least significant difference).[1]CFE= Coleus forskohlii roots extract. [2]IL2=interleukin 2. [3]IL3=interleukin 3. [4]IgG= immuoglobin G. [5]IgA= immuoglobin A.

Androgen (Testosterone, dehydroepiandrostrone or androstenedione) structure is determinant in either inhibiting or enhancing T lymphocyte proliferation, but testosterone was more potent than dehydroepiandrosterone in suppression of the thymocyte proliferation [31]. The present findings of significantly decreased total leukocytic count, frequencies of circulating CD^+4 and CD^+8 cells in hyperandrogenized rats, were in agreement with the previous reported results [27,31,32], suggesting an induction of oxidative stress caused by androgen that resulted in an increase of the nitric oxide synthase and nitric oxide, consequently lead to inhibition of the T cell proliferation. Additionally, steroids may forestall the production of regulatory T cells [5]. Treatment hyperandrogenized rats with CFE may be resulted in regulation of reactive oxygen species and the induction of AMP activated protein kinase (AMPK) of T lymphocytes [33,34].

The present study revealed a decreased IL-2 and IgG concentration in rats with androgen induced PCOS that was restored to control levels after CFE treatment. This result was consistent with the previous studies that have shown that dehydro-epiandrosterone (a week androgenic steroid) significantly reduced the Th_1 cytokines IL-2 and had no effect on the production of the Th_2 cytokine IL-4 in vitro after stimulation with the mitogens conconavalin A [35]. However, Meikle *et al*. [36] indicated that androgen is a steroid hormone that is directly involved in the regulation of IL-2 production by both normal and some T cell hybridomas. Th_1 (make IL-2 and IFN-ɣ) and Th_2 (make IL4, IL5 and IL6) cells are using different transmission pathways after T cell receptor (TCR) mediated stimulation. The protein kinase C pathway is the major system of activation in Th_1 cells, while different second messengers are generated in Th_2 cells after activation [37]. The increased IL-2 and IgG in CFE treated hyperandrogenized rats in the present study may be explained an increase of opioid peptides such as β-endorphin that affect both free intra-cellular calcium and intracellular cAMP concentrations [38]. The rise in intracellular calcium is dependent on calcium released from intracellular stores [39], in response to the generation of inositol 1,4.5-triphasphate [40] and the influx of extracellular calcium [39]. Interestingly, forskolin could increase calcium influx through voltage–gated channels [41]. Intracellular calcium mobilization is an important early event involved in T cell activation and proliferation [42] and both types of T cell were able to stimulate B cells to secrete antibodies like IgG [43]. Paradoxically, Muñoz et al., [37] recorded that forskolin

inhibited T cell receptor-mediated IL-2 production and proliferation in Th_1 cells suggested their differential sensitivity to high levels of cAMP

Conclusion

The present data revealed that opioid and immune systems were impaired in hyperandrogenized rats (PCOS model) and the CFE treatment could restore most of these functions indicating its essential role in modulating both humoral and cellular immunity.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربي

الاستجابة الهرمونية والمناعية للعلاج بنبات القسط في انا ث الجرذان المحدث بها تجريبا متلازمة تكيس المبايض

هدى محمد لطفى عبدالله[١] ,منى محمد عبد الهادى[٢] ,ماجدة نعمت عبدالحميدعبدالعال[١], سحر نصر محمد [١]ى, وهيب دخيل الله محمد الحربى[٣], عادل عمر باحاذق[٣]

[١]معهد بحوث □حة الحيوان- معمل الزقازيق

[٢]قسم علم وظائف الاعضاء-كليةالطب البيطرى-جامعةالزقازيق-مصر

[٣]قسم علم وظائف الاعضاء-كلية الطب-جامعة ام القرى-مكة المكرمة-المملكة العربية السعودية

متلازمة تكيس المبايض (PCOS) هي واحدة من أكثر الأسباب شيوعا لضعف التناسل وقد يحدث هذا المرض نتيجة إضطرابات فى الهرمونات أوفى جهاز المناعة مع النشاط المفرط في الجهاز العصبي الودي. وقد أجريت هذه الد□اسة لبيان تأثير مستخلص جذو□ نبات القسط فى تعديل مستويات بعض الهرمونات وبعض المؤشرات المناعية فى الجرذان المعالجة بهرمون الأند□وجين لإحداث متلازمة تكيس المبايض. تم تقسيم عدد خمسون من إناث الجرذان غير الناضجة أولا إلى مجموعتين: مجموعة ضابطة ومجموعة حقنت تحت الجلد بهرمون الأند□وجين (سيدوتيستون) بجرعة٦٠ملجم/كليو جرام من وزن الجسم لمدة ٣٥ يوم لإحداث متلازمة تكيس المبايض. ثم تم تقسيم كل من المجموعتين إلى مجموعاتان فرعيتان. المجموعاتان الفرعيتان الاوليتان (أعطيت محلول ملحى وأعطيت ٢٥ملجم/كجم من وزن الجسم من مستخلص جذو□ نبات القسط) والمجموعاتان الفرعيتان الأخريتان (أعطيت سيدوتيستون و سيدوتيستون + مستخلص جذو□ نبات القسط بنفس الجرعات السابقة) لمدة ٣ أسابيع. ثم تم تجميع عينات الدم عند نهاية التجربة وتم تقديرمستويات هرمون كو□تيكستو□ن والبيتا إندو□فين والمجموع الكلى لخلايا الدم البيضاء ومجموعات التمايز (CD^{+} 4)و(CD^{+} 8) وانترلوكين ٢ وانترلوكين ٣ وانترلوكين ٤ والأجسام المناعية G وA وقدأظهرت النتائج إنخفاض ملحوظ في تركيزالبيتا إندو□فين والمجموع الكلى لخلايا الدم البيضاء ومجموعات التمايز (CD^{+} 4) و(CD^{+} 8) وانترلوكين ٢ والأجسام المناعية G بينما زاد مستويات هرمون كو□تيكستو□ن وانترلوكين٤ فى جرذان متلازمة تكيس المبايض المعالجة بهرمون الأندو□جين. فى حين اظهرت جرذان متلازمة تكيس المبايض المعالجة بهرمون الأندو□جين + مستخلص جذو□ نبات القسط قيم طبيعية لمعظم المؤشرات ماعدا مستوى هرمون كو□تيكستو□ن مما يشير إلى دو□ مستخلص جذو□ نبات القسط فى تعديل مستويات المناعةمما يساعد فى تقصير مدة علاج متلازمة تكيس المبايض.