Metabolic Changes and Hormonal Disturbances in Polycystic Ovarian Syndrome Rats and the Amelioration Effects of Metformin and/or Cinnamon Extraction

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Abstract: Polycystic ovarian syndrome (PCOS) is a heterogeneous endocrine disorder that affects about one in 15 women worldwide. It is a major disorder characterized by elevated levels of male hormones (androgens), acne and hirsutism. It can even cause insulin resistance, anovulation and infertility on prolong incidence of cysts. Because it is known that increasing insulin sensitivity in polycystic ovarian syndrome results in improved ovulatory function and decreased serum androgen concentrations. The objective of this study was to evaluate the ability of metformin or/and cinnamon to improve the disturbance occur in the clinical and biochemical parameters in an animal model of PCOS. The obtained results revealed a significant (p<0.05) increase in hormonal profile (17β-estradiol, progesterone, testosterone, dihyrotestosterone, LH, FSH) in PCOS rats than those in control ones. Furthermore, insulin, insulin resistance, lipid profile (cholesterol, triglycerides, leptin) and total oxidant capacity (TOC) were significantly elevated in PCOS rats compared with the control group. On the other hand, induction of polycystic ovarian syndrome in rats caused a significant (p<0.05) decrease in sex hormone-binding globulin (SHBG) and total antioxidant capacity (TAC) levels. When PCOS rats group was treated with metformin or/and cinnamon, considerable amelioration effects in all previous studied parameters were pronounced dependent on certain mechanisms which were discussed according to available recent researches.
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1.Introduction

Insulin resistance and compensatory hyperinsulinemia are risk factors for type II diabetes mellitus, dyslipidemia, hypertension and atherosclerosis, a constellation of findings termed the metabolic syndrome **(Nestler *et al.,* 1999)**. Both are also prominent features of the polycystic ovarian syndrome (PCOS), a disorder characterized by chronic anovulation and hyperandrogenism that affect women of reproductive age. In these women, hyperinsulinemia may contribute to the pathogenesis of polycystic ovarian syndrome by promoting abnormal androgen secretion and disrupting folliculogenesis and menstrual cyclicity **(Azziz, 2003 and Feng *et al.,* 2013)**. Presumably, this occurs as a result of insulin stimulating testosterone production by the ovarian cell responsible for androgen biosynthesis, namely the thecal cell. There is a strong association between hyperandrogenaemia, an increase in the free androgen index and the metabolic syndrome in premenopausal women with or without full-blown PCOS **(Cussons *et al.,* 2008)**. Moreover, among obese hyperandrogenic adolescents (hyperandrogenaemia) were found to be a significant predictor of the metabolic syndrome, independent of obesity and insulin resistance **(Coviello *et al.,* 2006)**.

The traditional pharmacological therapy for patients with PCOS mainly addresses correction of the hyperandrogenic state and anovulation, but always neglects the associated long-term metabolic risks (i.e.; insulin resistance). Recently, the increasing evidence has shown that insulin resistance has an important implication in the pathogenesis of PCOS and the use of insulin-sensitizing drugs is an effective therapeutic approach. Metformin (The insulin-sensitizing drug) has been shown to ameliorate insulin resistance, decrease serum free testosterone, increase serum sex-hormone binding globulin and improve ovulatory frequency in women with PCOS **(Lord *et al.,* 2003)**. In both obese and non-obese PCOS patients, hyperandrogenism was effectively treated by reducing hyperinsulinemia using metformin **(Ganie *et al.,* 2004)**. In ovarian theca cells, metformin inhibits androstenedione production with no effect on progesterone **(Harborne *et al.,* 2005)**. Clinically, metformin therapy resulted in a significant decrease in the total serum testosterone **(Ertunc *et al.,* 2005)**. Moreover, metformin corrects not only ovarian hyperandrogenism but also functional adrenal hyperandrogenism in adolescents with PCOS **(Arslanian *et al.,* 2002)**.

Cinnamon is the bark of *Cinnamoni cassiae* and has been used as traditional folk herbs. The polyphenol

type-A polymers, procyanidin, extracted from cinnamon stimulates autophosphorylation of the insulin receptor and inhibits protein tyrosine phosphatase I. Adipocytes treated with cinnamon extract *in vitro* conditions increase the glucose uptake and glycogen synthesis by these two mechanisms (**Anderson *et al.*, 2004**). In *vivo*, cinnamon extract has been found to mitigate insulin resistance induced by high fructose diets in normal *Wister* rats as measured by the euglycemic clamp (**Qin *et al.*, 2004**). These findings suggested that cinnamon extract may potentiate insulin action by enhancing the insulin signaling pathways leading to increase phosphatidylinositol 3-kinase activity, which regulates insulin-stimulated glucose uptake and glycogen synthesis (**Qin *et al.*, 2003**).

The objective of this study was to evaluate the ability of metformin or/and cinnamon to correct hormonal profile and to increase sensitivity to insulin which would have beneficial effects on ovulation and ovarian production of androgens in female rats with the polycystic ovary syndrome.

2. Material and Methods

Fifty virgin adult female albino rats obtained from the Animal House of Sera and Antigens Center, Cairo, were used in this study. The animals weighing 150±10g were divided into two groups and housed five to a cage under standard conditions (21±2°C, 50-60% humidity and 12-hour light/12-hour dark cycle) for one week before and throughout the study, with free access to standard chow and tap water. The experimental protocols were approved in the Animal House of Nuclear Research Center, Inshas.

Experimental design:-

After one week of acclimatization, polycystic ovarian syndrome was induced in forty rats by intraperitoneal injection of 2mg 5α-dihydrotestosterone (DHT) dissolved in 0.2ml sesame oil /100g body weight/week for 8 weeks according to **Fassnacht *et al.* (2003)**. The drug solutions were made freshly at the beginning of each work. The dose of DHT (Sigma Chemical Co; USA) was chosen to reach to the hyperandrogenic state such as in women with PCOS, whose plasma DHT levels are approximately 6-8-fold higher than those of healthy controls (**Silfen *et al.*, 2003**). Control rats were treated with 0.2ml sesame oil/100g body weight/week for 8 weeks. After induction of PCOS (8 weeks); the animals were divided into 4 groups (10 rats for each) as follow:

- Polycystic ovarian syndrome rats group (PCOS): The animals in this group untreated for 15 and 30 days (5 rats in each interval) and served as PCOS rats group.

- Metformin rats group (PCOS + Metformin): The rats in this group treated with 2mg metformin /100g body weight/day *via* gastric tube for 15 and 30 days (5 rats in each interval). Metformin was purchased from Sigma Chemical Co; USA

- Cinnamon rats group (PCOS + Cinnamon): These animals received *via* gastric tube one ml of cinnamon extraction (5g commercial cinnamon boiled in 50ml distilled water for 15 minutes and left until cooled)/ 100g body weight/day for 15 and 30 days (5 rats in each interval) and served as PCOS + cinnamon rats group.

Mixture rats group (PCOS + Mix): These animals received a mixture from metformin and cinnamon as above described.

At the end of each experimental period (15 & 30 days), the rats were overnight fasted and killed by head decapitation. Blood were collected in clean dry test tubes to obtain sera for the determination of the sexual hormonal profile and biochemical parameters.

The concentrations of serum 17β-estradiol (E2), progesterone and testosterone were estimated by radioimmunoassay (RIA) using solid phase system. The kits were purchased from Isotops Ltd, Budapest, Hungary.

Sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating hormone (FSH), leptin and insulin concentrations were assayed by enzyme immunoassay techniques (EIA) by using specific kits for rats. The kits were purchased from IBL Co; Hamburg, Germany. However, the level of rat 5α-dihydrotestosterone was determined by ELISA (Sandwich immunoassay technique) using commercial kit (USCN-Life Science INC, China).

Serum cholesterol, triglycerides and glucose concentrations were determined colourimetrically using suitable kits (Human, Germany). Total antioxidant capacity (TAC) and total oxidative capacity (TOC)] levels were determined by the EIA kits (Labor Diagnostika Nord GmbH & Co.; Nordhorn, Germany).

The homeostatic model assessment (HOMA) value as a measure of insulin resistance (IR) was calculated using the following formula: fasting insulin (μU/L) × fasting glucose (mmol/L)/22.5 (**Matthews *et al.*, 1985**).

Determination of Statistical analysis:

Statistical differences between the means were assessed by analysis of variance (ANOVA) followed by Duncan's multiple range test according to **Duncan (1955)** and **Snedecor & Cochran (1982)** using a computer program (Costate). Values of $P < 0.05$ were considered statistically significant.

3.Results and Discussion

Polycystic ovarian syndrome (PCOS) has been considered a progressive multiglandular endocrinopathy where the delicate balance of the hypothalamic pituitary adrenal ovarian axis is disturbed, resulting in a failure of the cyclic

reproductive mechanism (**Hamilton *et al.*, 1995**). In addition, PCOS displays a number of metabolic abnormalities including hyperinsulinemia, insulin resistance, dyslipidemia and obesity (**Teede *et al.*, 2006**). In this study, rats injected by intraperitoneal injection of 2mg 5α-dihydrotestosterone (DHT) /100g body weight/week for 8 weeks to induce PCOS recorded a significant (p<0.05) increase in sexual hormonal profile (17-β estradiol, progesterone, testosterone, DHT, LH and FSH) (Table 1). These results may be due to the alterations in the pituitary sensitivity to gonadotropin releasing hormone (GnRH) stimulation rather than from dysfunctions of the hypothalamus pituitary adrenal axis or/and excess in the secretion of adrenocortical products and in response to ACTH stimulation. These results are in harmony with that obtained by **Oakley *et al.* (2011)** and **Mahood (2012)**.In regard to the mechanism of how hyperandrogenism may lead to ovulation defect and therefore formation of follicular cyst in the ovary, it has been proposed that elevated testosterone disrupts the regulation of GnRH secretion (**Kalro *et al.*, 2001**). In support of this event, either GnRH agonists or LH treatment induces ovulation in PCOS patients. A few animal studies reported that androgen suppresses progesterone receptor (PR) expression in the hypothalamus (**Lea *et al.*, 2001 and Ozawa, 2005**). As progesterone down-regulates GnRH secretion by activating progesterone receptors (PR) activity in thehypothalamus (**Pastor *et al.*, 1998**). However, androgen-mediated suppression of PR expression may ameliorate GnRH secretion and therefore LH secretion. This disturbance in the gonadotropins releasing hormone leads to defect in follicle development. This development may arrest outright leading to failure to ovulate (Anovulation) or be reduced leading to irregular ovulation (Oligo-ovulation). The arrested follicle may thus form a cystic structure, leading to the polycystic ovarian phenotype and loss of fertility in PCOS patients (**Oakley *et al.*, 2011**).

Table (1): Effects of supplementation of metformin or/and cinnamon on sexual hormonal profile in PCOS rats (Mean ± SE).

Groups	Control	PCOS	PCOS + Metformin	PCOS + Cinnamon	PCOS + Mix
17β-Estradiol (pg/ml)					
15 days N = 5	$3.762 \pm 0.067^{A}{}_{a}$	$10.582 \pm 0.209^{B}{}_{a}$	$7.193 \pm 0.165^{C}{}_{a}$	$8.573 \pm 0.182^{D}{}_{a}$	$6.386 \pm 0.141^{E}{}_{a}$
30 days N = 5	$3.817 \pm 0.091^{A}{}_{a}$	$12.017 \pm 0.253^{B}{}_{b}$	$6.404 \pm 0.147^{C}{}_{b}$	$7.362 \pm 0.169^{C}{}_{b}$	$4.952 \pm 0.127^{A}{}_{b}$
Progesterone (pg/ml)					
15 days N = 5	$1.941 \pm 0.058^{A}{}_{a}$	$3.759 \pm 0.119^{B}{}_{a}$	$2.802 \pm 0.084^{C}{}_{a}$	$3.217 \pm 0.105^{D}{}_{a}$	$2.361 \pm 0.073^{E}{}_{a}$
30 days N = 5	$1.885 \pm 0.056^{A}{}_{a}$	$4.814 \pm 0.131^{B}{}_{b}$	$2.254 \pm 0.075^{C}{}_{b}$	$2.932 \pm 0.088^{D}{}_{b}$	$2.071 \pm 0.068^{E}{}_{b}$
Testosterone (ng/ml)					
15 days N = 5	$0.363 \pm 0.011^{A}{}_{a}$	$0.747 \pm 0.039^{B}{}_{a}$	$0.647 \pm 0.027^{C}{}_{a}$	$0.652 \pm 0.029^{C}{}_{a}$	$0.565 \pm 0.026^{D}{}_{a}$
30 days N = 5	$0.358 \pm 0.013^{A}{}_{a}$	$1.088 \pm 0.052^{B}{}_{b}$	$0.557 \pm 0.023^{C}{}_{b}$	$0.561 \pm 0.025^{D}{}_{b}$	$0.489 \pm 0.022^{E}{}_{b}$
DHT (pg/ml)					
15 days N = 5	$15.825 \pm 0.142^{A}{}_{a}$	$104.872 \pm 1.861^{B}{}_{a}$	$75.104 \pm 1.174^{C}{}_{a}$	$83.102 \pm 1.368^{D}{}_{a}$	$64.348 \pm 1.082^{E}{}_{a}$
30 days N = 5	$16.076 \pm 0.148^{A}{}_{a}$	$125.241 \pm 2.327^{B}{}_{b}$	$48.753 \pm 0.831^{C}{}_{b}$	$59.757 \pm 0.971^{D}{}_{b}$	$33.023 \pm 0.452^{E}{}_{b}$
SHBG (nmol/L)					
15 days N = 5	$15.009 \pm 0.092^{A}{}_{a}$	$7.158 \pm 0.067^{B}{}_{a}$	$9.717 \pm 0.088^{C}{}_{a}$	$8.651 \pm 0.096^{D}{}_{a}$	$11.630 \pm 0.098^{E}{}_{a}$
30 days N = 5	$14.896 \pm 0.097^{A}{}_{a}$	$4.817 \pm 0.048^{B}{}_{b}$	$11.050 \pm 0.094^{C}{}_{b}$	$10.896 \pm 0.089^{D}{}_{b}$	$14.720 \pm 0.104^{E}{}_{b}$
LH (ng/ml)					
15 days N = 5	$3.962 \pm 0.079^{A}{}_{a}$	$7.641 \pm 0.267^{B}{}_{a}$	$6.873 \pm 0.214^{C}{}_{a}$	$6.891 \pm 0.221^{C}{}_{a}$	$5.946 \pm 0.185^{D}{}_{a}$
30 days N = 5	$3.873 \pm 0.082^{A}{}_{a}$	$9.191 \pm 0.318^{B}{}_{b}$	$5.296 \pm 0.174^{C}{}_{b}$	$6.017 \pm 0197^{D}{}_{b}$	$4.673 \pm 0.126^{B}{}_{b}$
FSH (ng/ml)					
15 days N = 5	$5.149 \pm 0.093^{A}{}_{a}$	$8.547 \pm 0.317^{B}{}_{a}$	$7.073 \pm 0.246^{C}{}_{a}$	$7.756 \pm 0.278^{D}{}_{a}$	$5.988 \pm 0.193^{E}{}_{a}$
30 days N = 5	$5.142 \pm 0.089^{A}{}_{a}$	$11.191 \pm 0.408^{B}{}_{b}$	$6.006 \pm 0.214^{C}{}_{b}$	$6.577 \pm 0.237^{D}{}_{b}$	$5.151 \pm 0.136^{A}{}_{b}$

- $^{A, B, C, D, E}$ Means with a common superscript within a row are significantly different (P<0.05).
- $^{a, b}$ Means with a common subscript within a column are significantly different (P<0.05).

Several recent reports conflict regarding the presence of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in women with PCOS. Anovulation is associated with disturbances in the feedback from the ovarian steroid hormones to the hypothalamus and pituitary, resulting in disturbances in the pulsatility of gonadotropin releasing hormone (GnRH) release. Gonadotropin-secretory changes, with a characteristic increase in LH relative to follicle stimulating hormone (FSH) release, have long been recognized in PCOS. It has also been suggested that the elevated concentrations of LH are due to an abnormal feedback by estrogen which may also induce thecal hyperplasia as LH stimulates theca cell proliferation (**Palaniappan & Menon, 2010, Oakley *et al.,* 2011; Mahood, 2012 and Feng *et al.,* 2013**).

Testosterone is the most important circulating androgen. Approximately one-half of a woman's serum testosterone is derived from peripheral conversion of secreted androstenedione, while the other half is derived from direct glandular secretion. The ovaries and the adrenal glands contribute equally to testosterone production in women, however, in PCOS the main source of androgens is thought to come from the ovaries (**Marshall, 2001**). Dysregulation of cytochrome P450C17, the androgen-forming enzyme in both the adrenals and the ovaries may be the central pathogenic mechanism underlying hyperandrogenism in PCOS. In the presence of 5-alpha-reductase, testosterone is converted within the cell to the more potent androgen dihydrotestosterone. Excess 5-alpha- reductase activity in the skin determines the presence or absence of hirsutism (**Plouffe, 2000**).

In contrast, PCOS rats group showed a significant ($P<0.05$) decrease in the level of sex hormone binding globulin (SHBG), another endocrine characteristic of PCOS (Table 1). This low level of SHBG is connected to a relative increase in unbound concentrations of androstenedione and testosterone, which is clinically related with hyperandrogenism of hirsutism (**McKenna, 1988**). These data are in parallel with those obtained by **Cibula *et al.* (2002)**. The last authors reported that SHBG was the most significant predictor of insulin sensitivity. Moreover, the strong negative relationship of fasting insulin with SHBG levels that has been reported by **Crave *et al.* (1995)**. They suggested that indeed insulin has inhibitory activity on liver SHBG production as shown by human hepatoma cell line *in vitro* studies and *in vivo* by peritoneal infusion of insulin in nonobese type I diabetes (**Lassmann-Vague *et al.*, 1994**). It has been proposed that SHBG may constitute an index of insulin resistance only in a hyperinsulinemic state (**Katsuki *et al.*, 1996**).

Androgens may both directly and indirectly result in alterations in glucose metabolism, ultimately causing a hyperinsulinemic state. Androgens may directly inhibit peripheral and hepatic insulin action. In the current study, PCOS rats group recorded a significant ($p<0.05$) elevation in the levels of serum glucose and insulin associated with a significant increment in the value of insulin resistance (HOMA-IR) as compared to their corresponding control group (Table 2). These results may be due to a defect in insulin binding caused by decreasing receptor number or their affinity or/and defects at the level of effect molecules such as glucose transporters and activities of their enzymes involved in glucose metabolism. A study by **Ciaraldi *et al.* (1992)** found that insulin receptor binding and kinase activity were intact in adipocytes of women with PCOS, although they exhibited marked decrease in insulin sensitivity for glucose transport stimulation. They concluded that there was a post-binding defect present, which was probably related to the increasing androgen levels in PCOS women. Also, the authors suggested that testosterone could induce insulin resistance in these women by reducing the number and efficacy of glucose transport proteins, specifically the type-4 glucose transporter (GLUT-4). GLUT-4 appears to be responsible for the insulin-related uptake of glucose in muscle and fat.

Insulin plays both direct and indirect roles in the pathogenesis of androgen excess in PCOS. Although, women with PCOS have peripheral insulin resistance associated with ovarian steroidogenesis appears to be hypersensitive to insulin **(Baillargeon & Nestler, 2006)**. Insulin acts synergistically with LH to enhance theca cell androgen production in women with PCOS by activating a specific signaling pathway *via* its own receptor **(Diamanti-Kandarakis *et al.,* 2008)**. In addition, insulin can stimulate human theca cell proliferation (**Duleba *et al.,* 1998)** and can also enhance ovarian growth and follicular cyst formation in rats (**Poretsky *et al.,* 1992)**. Hyperinsulinemia may also have adverse effects in women with PCOS through its action at non-ovarian sites including the liver, adrenal glands and pituitary **(Nestler, 1997)**. Moreover, insulin has an inhibitory effect on hepatic sex hormone binding globulin (SHBG) production in women with PCOS, increasing the proportion of biologically available androgens and thereby contributing to hyperandrogenism (**Crave *et al.,* 1995**). However, insulin potentiates ACTH-mediated adrenal androgen production **(Moghetti *et al.,* 1996)**. The concept that hyperinsulinemia affects GnRH pulse frequency and inappropriate gonadotropin secretion in PCOS by acting at pituitary level is mainly based on *in vitro* studies in which insulin has been shown to increase LH secretion from cultured rat

pituitary cells **(Adashi *et al.*, 1981)**. In contrast to animal studies, clinical investigations have not been able to demonstrate that insulin affects gonadotropin secretion in women with PCOS **(Moret *et al.*, 2009)**. However, acute administration of insulin in lean, normal young women increases LH pulse frequency, suggesting that there is a functional link between insulin and the activity of the hypothalamic-pituitary-ovary (HPO) axis **(Moret *et al.*, 2009).**

Although, the adipose tissue enlargement is suspected to result from an impaired glucose metabolism, the correlation between adiposity and insulin resistance in PCOS remains unclear. Adipocytes play an essential role in storing triglycerides, which provide energy in the form of free fatty acids (FFA) and the released FFA may contribute to the insulin resistance of peripheral tissues. In addition, adipocytes can secrete numerous peptides and cytokines, including resistin, TNF-α, adiponectin, leptin, etc. Adipocytes may confer effects on the systemic metabolism through these products, consequentially resulting in insulin resistance. The interaction between these products constitutes a complex regulatory net affecting the endocrine function of adipose tissues (**Mook *et al.*, 2004**).

In this study, intraperitoneal injection of 2mg 5α-dihydrotestosterone /100g body weight/week for 8 weeks to induce PCOS in rats caused a significant increment in serum total cholesterol, triglyceride, leptin and total oxidant capacity (TOC) as compared to their corresponding normal rats group. On the other hand, PCOS rats group showed decline in the serum total anti-oxidant capacity (Table 3). These results may be attributed to the excess of free radicals formation, deficiency in the auto-immune system, the disturbance in the hypothalamus-pituitary-thyroid (HPT) axis, the elevation of lipid absorption in the intestine associated with increment in it's anabolism in the hepatic cells, elevation in the hepatic *de novo* lipogenesis or/and the increment in the production of ceramides formation. These data are in harmony with those obtained by **Wang *et al.* (2010)**.

The adverse effects of androgen excess may be manifested in several systems. Androgen receptors are presented in adipocytes and testosterone has an anti-lipolytic effect on abdominal subcutaneous preadipocytes **(Andersson *et al.*, 2002)** apparently through selective inhibition of catecholamine-induced lipolysis **(Faulds *et al.*, 2003)**. Androgen excess would therefore be expected to produce fat accumulation and the abdominal obesity commonly found in PCOS, according to the above two studies of lean women with PCOS (**Andersson *et al.*, 2002** and **Faulds *et al.*, 2003)**. However, in obese PCOS women, the androgen action in adipocytes may differ from that observed in lean women with PCOS, as a decrease in androgens induced by a GnRH agonist in obese PCOS was shown to produce increases in visceral fat **(Dumesic *et al.*, 1998)**. Further, bioactive testosterone levels in obese PCOS correlated negatively with lipoprotein lipase activity and positively with catecholamine-stimulated lipolysis in subcutaneous abdominal adipocytes **(Rebuffe-Scrive *et al.*, 1989)**.

Table (2): Effects of supplementation of metformin or/and cinnamon on some carbohydrate parameters in PCOS rats (Mean ± SE).

Groups	Control	PCOS	PCOS + Metformin	PCOS + Cinnamon	PCOS + Mix
Fasting glucose (mmol/L)					
15 days N = 5	19.125±0.142 $^{A}{}_{a}$	31.392±0.861 $^{B}{}_{a}$	26.111±0.652 $^{C}{}_{a}$	28.985±0.723 $^{D}{}_{a}$	24.978±0.467 $^{E}{}_{a}$
30 days N = 5	19.176±0.145 $^{A}{}_{a}$	40.441±0.947 $^{B}{}_{b}$	22.329±0.311 $^{C}{}_{b}$	26.128±0.591 $^{D}{}_{b}$	21.559±0.202 $^{E}{}_{b}$
Fasting insulin (µU/mL)					
15 days N = 5	0.397±0.011 $^{A}{}_{a}$	0.718±0.039 $^{B}{}_{a}$	0.609±0.027 $^{C}{}_{a}$	0.681±0.031 $^{D}{}_{a}$	0.576±0.023 $^{E}{}_{a}$
30 days N = 5	0.394±0.010 $^{A}{}_{a}$	0.967±0.046 $^{B}{}_{b}$	0.532±0.021 $^{C}{}_{b}$	0.593±0.029 $^{D}{}_{b}$	0.457±0.018 $^{E}{}_{b}$
Insulin resistance (HOMA)					
15 days N = 5	3.375 ± 0.079 $^{A}{}_{a}$	10.018±0.217 $^{B}{}_{a}$	7.067±0.184 $^{C}{}_{a}$	8.772±0.196 $^{C}{}_{a}$	6.394±0.169 $^{D}{}_{a}$
30 days N = 5	3.336±0.082 $^{A}{}_{a}$	17.381±0.278 $^{B}{}_{b}$	5.279±0.149 $^{C}{}_{b}$	6.886±0163 $^{D}{}_{b}$	4.379±0.126 $^{B}{}_{b}$

- $^{A, B, C, D, E}$ Means with a common superscript within a row are significantly different (P<0.05).
- $^{a, b}$ Means with a common subscript within a column are significantly different (P<0.05).

Table (3): Effects of supplementation of metformin or/and cinnamon on some biochemical parameters in PCOS rats (Mean ± SE).

Groups	Control	PCOS	PCOS + Metformin	PCOS + Cinnamon	PCOS + Mix
Total cholesterol (mg/dL)					
15 days N = 5	57.762±1.067 $^{A}{}_{a}$	90.582±2.209 $^{B}{}_{a}$	72.193±1.165 $^{C}{}_{a}$	78.573±1.182 $^{D}{}_{a}$	66.386±1.141 $^{E}{}_{a}$
30 days N = 5	58.417±1.091 $^{A}{}_{a}$	118.017±2.853 $^{B}{}_{b}$	58.404±1.137 $^{C}{}_{b}$	65.362±1.159 $^{D}{}_{b}$	57.952±1.117 $^{A}{}_{b}$
Triglycerides (mg/dL)					
15 days N = 5	64.941±1.158 $^{A}{}_{a}$	103.759±2.619 $^{B}{}_{a}$	88.302±2.184 $^{C}{}_{a}$	93.217±2.305 $^{D}{}_{a}$	79.361±1.873 $^{E}{}_{a}$
30 days N = 5	66.085±1.156 $^{A}{}_{a}$	144.814±3.231 $^{B}{}_{b}$	72.254±1.175 $^{C}{}_{b}$	82.932±1.928 $^{D}{}_{b}$	65.071±1.168 $^{A}{}_{b}$
Leptin (pg/ml)					
15 days N = 5	0.397±0.042 $^{A}{}_{a}$	0.947±0.089 $^{B}{}_{a}$	0.677±0.077 $^{C}{}_{a}$	0.752±0.081 $^{D}{}_{a}$	0.594±0.068 $^{E}{}_{a}$
30 days N = 5	0.398±0.042 $^{A}{}_{a}$	1.228±0.112 $^{B}{}_{b}$	0.502±0.059 $^{C}{}_{b}$	0.662±0.071 $^{D}{}_{b}$	0.431±0.049 $^{E}{}_{b}$
Serum TAC (mmol/L)					
15 days N = 5	1.842±0.027 $^{A}{}_{a}$	0.469±0.019 $^{B}{}_{a}$	0.668±0.023 $^{C}{}_{a}$	0.591±0.024 $^{D}{}_{a}$	0.822±0.021 $^{E}{}_{a}$
30 days N = 5	1.838±0.028 $^{A}{}_{a}$	0.287±0.015 $^{B}{}_{b}$	0.813±0.022 $^{C}{}_{b}$	0.743±0.023 $^{D}{}_{b}$	1.275±0.025 $^{E}{}_{b}$
Serum TOC (mmol/L)					
15 days N = 5	0.358 ± 0.007 $^{A}{}_{a}$	1.094±0.023 $^{B}{}_{a}$	0.809±0.018 $^{C}{}_{a}$	0.941±0.019 $^{D}{}_{a}$	0.692±0.016 $^{E}{}_{a}$
30 days N = 5	0.366±0.008 $^{A}{}_{a}$	1.356±0.031 $^{B}{}_{b}$	0.725±0.014 $^{C}{}_{b}$	0.857±0.017 $^{D}{}_{b}$	0.497±0.011 $^{E}{}_{b}$

- $^{A, B, C, D, E}$ Means with a common superscript within a row are significantly different (P<0.05).
- $^{a, b}$ Means with a common subscript within a column are significantly different (P<0.05).

Leptin is produced by the human fat body stores and appears to be involved in the regulation of the reproductive axis. The increment in leptin level was be found in PCOS rats group when compared to control group may be a reflection to the increase of fat cell size and increased body fat mass (**Mendonca *et al.*, 2004**). **Sun & Yu (2000)** reported that the elevation of E2 levels in rats led to a marked increment in the leptin level and neuropeptide Y expression associated with a considerable decline in GnRH and gonadotropin secretion. Moreover, the alteration in the fat distribution is a consequence of steroid metabolism, but the mechanisms responsible are still unknown. Furthermore, increased adiposity in ovariectomized rats can be reversed with 17β-estradiol treatment (**Ainslie *et al.*, 2001**). In this animal model, the last authors suggested that obesity is not associated with hypoleptinemia or decreased *Ob* gene expression, but associated with insensitivity to central leptin administration caused by estrogen deficiency.

Estradiol may stimulate the production of leptin from the adipocytes (**Tanaka *et al.*, 2001**). Leptin could act in the pituitary ovarian axis during fasting to improve reproductive function by partly stimulating estrogen secretion. Thus, the role of leptin in PCOS may occur by ways other than the simple concentration of the hormone in the circulation and estrogen metabolism may be involved in this role (**Tanaka *et al.*, 2001**).

Decreased insulin sensitivity in PCOS women were accounted for by impaired rate of both glucose oxidation and non-oxidation. These data are similar with those obtained by **Dunaif *et al.* (1995)**. The authors noted that the defect in insulin sensitivity in PCOS may lie in excessive serine phosphorylation of the insulin receptor, which, in turn, inhibits the proximal intracellular insulin signalling cascade, leading to impair glucose oxidation and glucose non-oxidation. Also, the impairment in insulin sensitivity is mainly explained by the fact that excessive amounts of free fatty acids, used as substrates for lipid oxidation, competed with glucose in the muscle cells as a source of energy **(Ferrannini *et al.*, 1983).** Similarly, a tendency towards higher FFA concentrations and defective suppression of rate of lipid oxidation were found during the hyperinsulinaemic clamp in obese PCOS subjects which associated with abdominal obesity, insulin resistance and hyperandrogenism (**Holte *et al.*, 1995**).

Metformin, a biguanide antihyperglycemia drug, has been shown to improve hyperandrogenism and hyperinsulinemia, most likely through its positive

effects on glucose utilization in insulin-sensitive tissues **(Weerakiet *et al.*, 2004)**. In the current work, PCOS rats treated by metformin recorded an improvement in all parameters studied (Tables 1, 2 & 3). These results may be due to the pharmakinitics and pharmadynamics of metformin which can be acted as steroidogenic agent (has the ability of ovarian stimulation by the GnRH agonist and leuprolide acetate). These results are in harmony with those obtained by **Velazquez *et al.* (1994).** The authors reported that metformin treatment ameliorated the hyperinsulinemia and hyperandrogenemia of PCOS. Moreover, insulin appears to modulate the 17-hydroxylase and 17,20-lyase activities of the ovarian steroid-forming P450C17. This enzyme is characteristically abnormally regulated in women with the ovarian androgen excess of PCOS as reflected in the 17-hydroxyprogesterone response to GnRH agonists, including nafarelin, buserelin and leuprolide (**Ehrmann *et al.*, 1997**).

In both obese and non-obese PCOS patients, hyperandrogenism was effectively treated by reducing hyperinsulinemia using metformin (**De Leo *et al.*, 2003**). In ovarian theca cells, metformin inhibits production of androgens through reducing pituitary secretion of LH, leading to ovulation and regular menstrual cycles. Also, metformin inhibits androstenedione production with no effect on progesterone (**Harborne *et al.*, 2005**). Clinically, metformin therapy resulted in a significant decrease in the total serum testosterone (**Harborne *et al.*,, 2005**). Moreover, metformin corrected not only ovarian hyperandrogenism but also functional adrenal hyperandrogenism in adolescents with PCOS (**Wang, 2006**).

Recent evidence suggested that one of the modes of action of metformin may be through phosphorylation of the insulin receptor and insulin receptor substrates (**Ertunc *et al.*, 2005**). In addition, metformin appears to induce cardioprotective effects on plasminogen activator inhibitor (PAI)-1 as well as serum lipids by decrease the release of free FFAs from adipose tissue (**Wang, 2006**). Decreasing competition between serum glucose and FFAs as energy substrates in peripheral tissues could result in an improvement of glucose oxidation and consequently insulin sensitivity and hyperinsulinemia (**Morin-Papunen *et al.*, 2000**).

The supplementation of aqueous extracts of cinnamon to the PCOS rats led to a considerable correction in all studied parameters dependent on the time of supplementation. These results were confirmed by **Roussel *et al.* (2009)**. They reported that polyphenol type-A polymers extracted from cinnamon stimulates autophosphorylation of the insulin receptor and inhibits protein tyrosine phosphatase (PTP-1). Both these mechanisms may lead to increase glucose uptake and glycogen synthesis. Cinnamon extract may potentiate insulin action *via* enhancing the insulin signaling pathways leading to increased PI 3-kinase activity, which regulates insulin-stimulated glucose uptake and glycogen synthesis. Cinnamon extract has also been found to mitigate insulin resistance as measured by the euglycemic clamp when induced by a high fructose diet in normal *Wistar* rats **(Qin *et al.*, 2004)**.

Furthermore, cinnamon polyphenols activate insulin receptors by increasing the amount of insulin receptor b and GLUT4 protein. Cinnamon increases glycogen synthase activity and glycogen accumulation with decreases glycogen synthetase kinase-3b activity. Also, it increases the amount of the early response anti-inflammatory protein and tristetraprolin. All these activities and other potential activities may eventually lead to more efficient glucose transport and utilization. In addition, cinnamon polyphenols induced tristetraprolin accumulation may provide one of the molecular bases for the beneficial effects of cinnamon in improving the conditions of individuals with metabolic syndrome and insulin resistance by down regulating the synthesis of pro-inflammatory cytokines (**Cao *et al.*, 2007**).

Cinnamon has been reported to improve the antioxidant status of subjects with the metabolic syndrome. **Roussel *et al.* (2006)** showed that plasma malondialdehyde levels were reduced by the aqueous extract of cinnamon, indicating decreased lipid peroxidation, while plasma SH groups were increased, indicating a protection of antioxidant SH groups against oxidation. The authors attributed these data due to the excess of the glutathione pool in the liver, increment in the enzymes antioxidant activities such as catalase (CAT), superoxide dismutase (SOD) and xanthine oxidase (XOD) or/and enhancement in the hepatic *de novo* lipogenesis. In the group receiving cinnamon, plasma SH groups were found to be increased after 12 weeks of supplementation, suggesting that cinnamon acts in protecting both lipids and proteins against oxidation.

Also, cinnamon extracts inhibited retinol-binding protein-4 (RBP-4), a novel adipokine that contributes to insulin resistance in plasma and adipose tissues. Retinol-binding protein 4 is increased in the serum of insulin-resistant humans and rodents and mediates insulin resistance in muscle and increased glucose production in liver (**Polonsky, 2006**).

From the above cited data, it could be concluded that endocrine and metabolic factors, including insulin resistance, obesity and hyperandrogenaemia appear to contribute to the development of anovulation in polycystic ovarian syndrome cases. Metformin alone or associated with cinnamon improve (s) the disorders caused by PCOS to a significant extent including

amelioration of hyperinsulinemia, dyslipidemia and hyperandrogenism and offers a good treatment alternative for anovulation.

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