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/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, dihydrotestosterone, 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/cinnamon, considerable amelioration effects in all previous studied parameters were pronounced dependent on certain mechanisms which were discussed according to available recent researches.

Key words: Polycystic Ovarian syndrome, Metabolic Changes, Hormonal Disturbance, Metformin, Cinnamon.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>PCOS</th>
<th>PCOS + Metformin</th>
<th>PCOS + Cinnamon</th>
<th>PCOS + Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>17β-Estradiol (pg/ml)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>3.762 ± 0.067IAM</td>
<td>10.582 ± 0.209Ib</td>
<td>7.193 ± 0.165Ia</td>
<td>8.573 ± 0.182Ib</td>
<td>6.386 ± 0.141Ia</td>
</tr>
<tr>
<td>30 days N = 5</td>
<td>3.817 ± 0.091IAM</td>
<td>12.017 ± 0.253Ib</td>
<td>6.404 ± 0.147Ib</td>
<td>7.362 ± 0.169Ib</td>
<td>4.952 ± 0.127Ib</td>
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<td><strong>Progesterone (pg/ml)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>1.941 ± 0.058IAM</td>
<td>3.759 ± 0.119Ib</td>
<td>2.802 ± 0.084Ia</td>
<td>3.217 ± 0.105Ib</td>
<td>2.361 ± 0.073Ia</td>
</tr>
<tr>
<td>30 days N = 5</td>
<td>1.885 ± 0.056IAM</td>
<td>4.814 ± 0.131Ib</td>
<td>2.254 ± 0.075Ib</td>
<td>2.932 ± 0.088Ib</td>
<td>2.071 ± 0.068Ib</td>
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<td><strong>Testosterone (ng/ml)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>0.363 ± 0.011IAM</td>
<td>0.747 ± 0.039Ib</td>
<td>0.647 ± 0.027Ib</td>
<td>0.652 ± 0.029Ib</td>
<td>0.565 ± 0.026Ib</td>
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<tr>
<td>30 days N = 5</td>
<td>0.358 ± 0.013IAM</td>
<td>1.088 ± 0.052Ib</td>
<td>0.557 ± 0.023Ib</td>
<td>0.561 ± 0.025Ib</td>
<td>0.489 ± 0.022Ib</td>
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<td><strong>DHT (pg/ml)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>15.825 ± 0.142IAM</td>
<td>104.872 ± 1.861Ib</td>
<td>75.104 ± 1.174Ia</td>
<td>83.102 ± 1.368Ib</td>
<td>64.348 ± 1.082Ia</td>
</tr>
<tr>
<td>30 days N = 5</td>
<td>16.076 ± 0.148IAM</td>
<td>125.241 ± 2.327Ib</td>
<td>48.753 ± 0.831Ib</td>
<td>59.757 ± 0.971Ib</td>
<td>33.023 ± 0.452Ib</td>
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<tr>
<td><strong>SHBG (nmol/L)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>15.009 ± 0.092IAM</td>
<td>7.158 ± 0.067Ib</td>
<td>9.717 ± 0.088Ia</td>
<td>8.651 ± 0.096Ib</td>
<td>11.630 ± 0.098Ia</td>
</tr>
<tr>
<td>30 days N = 5</td>
<td>14.896 ± 0.097IAM</td>
<td>4.817 ± 0.048Ib</td>
<td>11.050 ± 0.094Ib</td>
<td>10.896 ± 0.089Ib</td>
<td>14.720 ± 0.104Ia</td>
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<td><strong>LH (ng/ml)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>3.962 ± 0.079IAM</td>
<td>7.641 ± 0.267Ib</td>
<td>6.873 ± 0.214Ia</td>
<td>6.891 ± 0.221Ia</td>
<td>5.946 ± 0.185Ia</td>
</tr>
<tr>
<td>30 days N = 5</td>
<td>3.873 ± 0.082IAM</td>
<td>9.191 ± 0.318Ib</td>
<td>5.296 ± 0.174Ib</td>
<td>6.017 ± 0.019Ib</td>
<td>4.673 ± 0.126Ib</td>
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<tr>
<td><strong>FSH (ng/ml)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>5.149 ± 0.093IAM</td>
<td>8.547 ± 0.317Ib</td>
<td>7.073 ± 0.246Ia</td>
<td>7.756 ± 0.278Ib</td>
<td>5.988 ± 0.193Ia</td>
</tr>
<tr>
<td>30 days N = 5</td>
<td>5.142 ± 0.089IAM</td>
<td>11.191 ± 0.408Ib</td>
<td>6.066 ± 0.214Ib</td>
<td>6.577 ± 0.237Ib</td>
<td>5.151 ± 0.136Ia</td>
</tr>
</tbody>
</table>

Means with a common subscript within a column are significantly different (P<0.05).

Means with a common superscript within a row 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 (Moggetti 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 observed 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).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>PCOS</th>
<th>PCOS + Metformin</th>
<th>PCOS + Cinnamon</th>
<th>PCOS + Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
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<tr>
<td>15 days N = 5</td>
<td>19.125±0.142 A a</td>
<td>31.392±0.861 B a</td>
<td>26.111±0.652 C a</td>
<td>28.985±0.723 D a</td>
<td>24.978±0.467 E a</td>
</tr>
<tr>
<td>30 days N = 5</td>
<td>19.176±0.145 A a</td>
<td>40.441±0.947 B b</td>
<td>22.329±0.311 C b</td>
<td>26.128±0.591 D b</td>
<td>21.559±0.202 E b</td>
</tr>
</tbody>
</table>

| **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 D a | 6.394±0.169 E a |
| 30 days N = 5 | 3.336±0.082 A a | 17.381±0.278 B a | 5.279±0.149 C b | 6.886±0163 D b | 4.379±0.126 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 hypoletinemia 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 Dunai 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

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

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

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

References
33. Lea, R.; Clark, J. and Tsutsui, K.: Changes in central steroid receptor expression, steroid synthesis and dopaminergic activity