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Tri-Chromium picolinate significantly improved the hormonal levels and CYP17A1 expression in female rats induced with polycystic ovary syndrome

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Abstract

Introduction: Polycystic ovary syndrome is the most common condition of endocrine and metabolic issues in women of reproductive age. It is associated with androgen excess, insulin resistance, and enlarged ovaries. Tri-Chromium picolinate is a salt of the chemical element chromium. It's effectively treating hyperinsulinemia and hyperlipidemia.

Objectives: The investigation sought to assess the effects of different concentrations of Tri-Chromium picolinate on the hormonal levels and the expression of cytochrome 17A1 in the female rats induced with polycystic ovary syndrome.

Materials and Methods: Forty-eight female albino rats were used in this experimental study design. Rats were divided into six groups, with eight animals in each group. All groups were given testosterone enanthate 100 mg/kg/d by subcutaneous injection for 28 days, while the control group was given sesame oil 0.5 ml for 28 days. In the treatment stage, Tri-Chromium picolinate (1 mg, 2 mg, and 4 mg/kg/d) was given to groups III, IV, and V, respectively, for 42 days, and cyproterone acetate was given for 42 days to group VI for comparison. At the same time, the control and induction groups were treated with distilled water 0.5 mL orally for 42 days. In the current study, the serum was stored until used for determining serum marker levels. Meanwhile, the ovaries were harvested for immunohistochemical examination to assess the effect of Tri-Chromium picolinate on Cytochrome P45017A1 expression.

Results: Tri-Chromium picolinate significantly improved the hormonal levels (testosterone, free. testosterone, luteinizing hormone, follicle-stimulating hormone), it also significantly decreases androgen synthetic pathway (androstenedione and aldo-keto reductase) and downregulated Cytochrome P45017A1 expression in a dose-dependent manner.

Conclusion: Tri-Chromium picolinate exhibits anti-androgenic activity by improving hormonal levels and downregulating the expression of cytochrome P450 17A1.

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Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common conditions associated with the endocrine system of women of reproductive age (1). Defects in the action of insulin and the function of the hypothalamus-pituitary are the causes of PCOS (2). Evidence supports the role of several internal and external factors, including genetics, epigenetics, hyperandrogenism, insulin resistance (IR), and environmental factors. The long-term effects of this condition include increased testosterone and obesity, insulin resistance, diabetes type 2, and oxidative stress (3-5). These patients have a lower release of gonadotropin in conjunction with a higher secretion of luteinizing hormones (LH) than follicle-stimulating hormones (FSH) (6). Similarly, IR increases the activity of Cytochrome P450 17A1 (CYP17A1), the enzyme that produces androstenedione and testosterone (7). On the other hand, hyperinsulinemia is associated with increased testosterone levels in the blood by decreasing the amount of sex hormone-binding globulin in the liver (7,8). Polycystic ovary syndrome cannot be diagnosed using normal diagnostic procedures like blood tests, cultures, or biopsies due to the lack of a particular test for its diagnosis. Differential diagnosis involves excluding relevant disorders based on symptoms and limited options. To confirm a differential diagnosis for PCOS, it is important to rule out hyperprolactinemia, thyroid disease, Cushing's syndrome, and adrenal hyperplasia based on the linked examinations

Key point

It is crucial to find safe and effective drugs that operate on different pathways to present a new approach to treating polycystic ovary syndrome to minimize the adverse effects associated with the available polycystic ovary syndrome drugs. Tri-Chromium picolinate, a safe supplement, effectively treats hyperinsulinemia and hyperlipidemia. The results in this study revealed that Tri-Chromium picolinate significantly improved the hormonal levels and decreased the expression of the cytochrome P45017A1 enzyme; therefore, Tri-Chromium acts on different pathways (insulin resistance, hyperlipidemia, and hyperandrogenemia) in polycystic ovary syndrome.

(9). Many complications are linked to PCOS, including long-term ones such as endometrial cancer, breast cancer, obesity, diabetes, cardiovascular risk, and short-term ones such as hirsutism, acne, obesity, infertility, dyslipidemia, and glucose tolerance (10) as well as pregnancy outcomes, which include fetal growth abnormalities, gestational diabetes, preeclampsia, premature birth, and spontaneous abortion (11). Tri-Chromium picolinate is a salt of the trace metallic element chromium. This supplement improves insulin signaling by reducing membrane cholesterol levels, hence increasing glucose transportation within cells (12,13). Chromium is regarded as one of fifteen trace elements essential for the proper physiological functioning of lipid and carbohydrate metabolism. A lack of it has been associated with a variety of diseases, including the symptoms of diabetes type 2 and heart disease (14). The toxicity of chromium is linked to the hexavalent form, which is 100 times more harmful when consumed than the trivalent form (15). Animal studies have failed to demonstrate a toxic effect of chromium at high doses of 15 mg/kg body weight (16). Human clinical trials failed to demonstrate any objective toxicity or abnormalities in the liver or kidneys at 1000 mcg daily for four months in diabetes or at 500 mcg after a year (17).

Objectives

To investigate the effect of different doses of Tri-Chromium picolinate on the testosterone, free testosterone, androstenedione, aldo-ketoreductase, LH, FSH, and thyroid stimulating hormone (TSH) levels and expression of Cyp17A1 in polycystic ovary syndrome-induced rats and to determine whether Tri-Chromium picolinate is affecting PCOS by decreasing the hyperandrogenism secondary to its effect on serum glucose or the Tri-Chromium picolinate has a direct effect on steroidogenic enzymes hence treating PCOS through directly reducing testosterone.

Materials and Methods

Study design

Materials used in this study include Tri-Chromium picolinate and cyproterone acetate powder from TCI Japan, testosterone enanthate ampule (250 mg/mL) from Panpharma, Germany, solvent (tween 80) from research product international RPI, USA, distilled water from pioneer, Iraq, xylazine vial (20 mg/ml) from Kepro, Holland, Ketamine vial (10 %) from Alfasan, Holland, hematoxylin and eosin from Sigma, Germany.

For this experimental animal study, 54 female albino rats (Rattus norvegicus) were purchased from local stores, their ages were 21 days. These animals were maintained in a well-ventilated container and were permitted to have free access to water and food at a temperature of 25 ±2°C in natural light/dark cycles with a relative humidity of 55.0%. The rats were given a week to acclimate to the animal house before initiating the experiment. The study proceeded for 70 days. A pilot study was carried out to confirm the PCOS induced by testosterone enanthate. The animals were randomly assigned to six groups, with each group consisting of eight animals.

- Group I (healthy control group): Eight female rats received only 0.5 mL of the sesame oil once daily by subcutaneous (S.C) for 28 days (induction period), and then 0.5 mL distilled water (D.W) and tween (80) once daily by oral gavage syringe for 42 days (treatment period).
- Group II (induction group): Eight female rats received daily doses of testosterone enanthate 1 mg/100 g body weight (B.W)/day by S.C for 28 days then received only 0.5 mL D.W and tween (80) once daily by oral gavage syringe for 42 days.
- Group III (Low dose of Tri-Chromium picolinate group, LDTCP): Eight female rats received daily doses of testosterone enanthate (1 mg/100 g B.W/day) as S.C for 28 days, then received Tri-Chromium picolinate dose (1 mg/kg) once daily in D.W and tween 80 orally for 42 days.
- Group IV (Moderate dose of Tri-Chromium picolinate group, MDTCP): Eight female rats received daily doses of testosterone enanthate (1 mg/100 g B.W/day) by S.C for 28 days, then received Tri-Chromium picolinate dose (2 mg/kg) once daily in D.W and tween 80 orally for 42 days.
- Group V (High dose of Tri-Chromium picolinate group, HDTCP): Eight female rats received daily doses of testosterone enanthate (1mg/100 g B.W/day) by S.C for 28 days, then received Tri-Chromium picolinate dose (4 mg/kg) once daily in D.W and tween 80 orally for 42 days.
- Group VI (standard group): Eight female rats received daily dose of testosterone enanthate (1 mg/100 g B.W/day) by S.C for 28 days, then received cyproterone acetate (2 mg/d) once daily in D.W and tween 80 orally for 42 days.

Preparation of drug and doses

The administration of the dose was conducted at 8:30-9:30 AM every day to avoid hormonal changes that may negatively affect the rat's estrus cycle. The induction of

PCOS was accomplished by administering testosterone enanthate at a concentration of 1 mg/100 g B.W./day S.C. at the neck's dorsum for 28 days (18), the preparation of the substance was conducted by diluting testosterone enanthate into 24 mL of sesame oil, which resulted in each ml having 10 mg of the substance. The concentrations of Tri-Chromium picolinate in the dosages were selected based on a literature review (19,20), the compound was prepared by dissolving 15 mg of the pure drug (i.e. without any additives) in 15 mL of distilled water and tween 80, which each 1 mL of the mixture contained 1 mg of the drug. It was administered by oral gavage in a dosage of a concentration of 1 mg/kg/d, 2 mg/kg/d, and 4 mg/kg/d for 42 days.

Cyproterone acetate powder was administered for comparison at a dose of 2 mg/d (21) by oral gavage for 42 days once daily, it was prepared by dissolving 30 mg of the pure drug in 3 ml of distilled water and tween 80. An insulin syringe was used to withdraw the exact concentration. Therefore, each 10 units contains 1 mg of cyproterone acetate.

Sample collection

At the end of the experiment, the rats were permitted to fast for 12 hours, and under anesthesia with ketamine and xylazine injections intraperitoneal at 100 and 10 mg/kg, respectively, a 5-cc syringe was employed to draw blood directly from the heart by piercing the right ventricle. The blood was collected in a gelatinous tube that would allow the serum to be separated by centrifuging at 1000 RPM for 15 minutes. The serum was aspirated by micropipettes, transferred to labeled Eppendorf tubes, and stored at a temperature of -20 °C until it was used to measure the serum levels of the markers (22).

Measurements of serum biomarkers

The reading was done after 70 days of starting the experiment to compare biomarker levels among groups. These biomarkers include hormonal tests include serum total and free testosterone, FSH, LH, TSH, and androgen synthetic pathway markers; aldo-keto reductase type 1C3 (AKR1C3) and androstenedione. The serum hormonal levels were determined by using the enzyme-linked immunosorbent assay technique, and the procedure was performed according to the manufacturer's guidelines (23).

Ovarian harvest

The ovaries were harvested and collected in formalin (10%) for immunohistochemical examination to assess the effect of Tri-Chromium picolinate on Cyp17A1 expression.

Immunohistochemistry

The immunohistochemistry (IHC) method allows for the visualization of the location and distribution of specific cellular components in a tissue. Employing the recommended procedure by Panzan et al (24), the results were categorized by using a colorimetric system that depended on the degree to which the tissue was brownish.

The cells that were stained were classified into several classes based on the percentage of cells in a typical area. Grade 0 represented 0% of stained cells, grade 1 represented 1% to 25% of stained cells, grade 2 represented 26% to 50% of stained cells, grade 3 represented 51% to 75% of stained cells, and grade 4 represented 76% to 100% of stained cells. The immunoreactions' reactions were assessed based on their intensity, which was categorized as negative (0), mild (I), moderate (II), or strong/intense (III). The sections that lacked any coloring were considered negative.

The total index was determined using the following formula:

Total index = percentage of cells that are immunoreactive × the intensity of their immunoreaction. The results are described as numbers from 0 to 12 (24).

Procedure

After ovary tissue fixation with 10% neutral buffered saline, the samples were embedded in paraffin wax for storage and to enable sectioning. Ovarian tissues for IHC were sectioned into 4 μM slices with a microtome and then mounted onto charged microscope slides. The charged slides were heated in a tissue-dry oven at 60 °C for 45 minutes. Deparaffinization was the next step including: Immerse the slides in xylene for 5 minutes, changing the xylene three times. Then, rinse the slides with three changes of 100% ethanol. Each step should be performed for a duration of 3 minutes, followed by two subsequent changes using a 95% ethanol solution. Each step requires a duration of 3 minutes, and the final step involves replacing 80% of the ethanol for a duration of 3 minutes. Thoroughly wash the slides by placing them under a mild stream of distilled water for a duration of 5 minutes. After removing the wax, the antigen is retrieved by placing the slides in a solution of 0.01 M sodium citrate buffer with a pH of 6.0. The slides are then heated to a temperature of 99–100 °C for a duration of 20 minutes using steam in order to remove crosslink proteins to help the antibody penetrate antigens in specific tissues. After that, add 3% hydrogen peroxide to slides in order to prevent non-specific binding of antibodies to tissue. Labelled antibody is added to slides. Antibody-antigen (Ab-Ag) conjugation was visualized by adding labeled enzyme. In presence of chromogenic substrate (diaminobenzidine), the labeled peroxidase enzyme produces brown color precipitate at Ab-Ag binding site. In order to improve the visualization of slides, a hematoxylin stain is added to contrast the primary stain by giving the tissue a blue color. After all staining process is completed, the slides must be preserved for long-term usage by adding coverslips with appropriate mounting medium (25). The slides were viewed by light microscope under supervision of professional pathologist.

Statistical analysis

The mean plus or minus the standard error of mean was used to describe the data in this study. The Statistical Package for the Social Sciences (SPSS) version 25.0 was employed for the statistical analysis. The analysis of variance (ANOVA) procedure and the post hoc Tukey test were employed to determine the degree to which different means were significant. The Kruskal-Wallis test and Mann-Whitney test were employed to evaluate the IHC results, which did not exhibit a normal distribution. Significant, highly significant, and very highly significant differences were considered to have a *P* value of less than 0.05, 0.01, and 0.001, respectively.

Results

Regarding the effect on hormonal markers after treatment with Tri-Chromium picolinate, the mean serum testosterone level was increased to a very significant degree in the induction group compared to the other groups. The average serum testosterone level in the treatment groups (MDTCP, HDTCP, and standard) was drastically reduced when compared to the LDTCP group. Meanwhile, the level of the HDTCP marker was significantly higher than that of the control group; however, it did not differ significantly from the standard group. In contrast, the levels of the LDTCP and MDTCP groups were significantly higher than those of both the control and standard groups. The effect of the HDTCP group on this marker was similar to the standard group, as shown in Figure 1 and Table 1.

Concerning the effect on free testosterone levels following treatment with Tri-Chromium picolinate, the mean serum level of this hormone significantly increased in the induction group compared to the other groups. In contrast, the mean serum level of free testosterone in the HDTCP group significantly decreased compared to the LDTCP group. The effects of the MDTCP and HDTCP groups on this marker were highly significant in comparison to the control group. However, the average serum level of free testosterone in the MDTCP group did not differ significantly from that in the HDTCP group, as illustrated in Figure 1 and Table 2.

Regarding the effect of treatment on FSH, the mean serum FSH level significantly decreased in the induction group compared to the other groups. Conversely, the average serum FSH levels in the treatment groups (LDTCP, MDTCP, and HDTCP) were significantly higher than those in the induction group, exhibiting a dose-dependent relationship. Additionally, the average serum FSH levels in the treatment groups (LDTCP and MDTCP) were significantly greater than those in the control and standard groups. Similarly, the impact of the HDTCP group on these marker traits was greatly enhanced in comparison to the control group. However, there was no significant difference between the HDTCP and standard groups; the effect of HDTCP on the concentration of this marker was almost identical to the standard group. Additionally, the standard group's marker levels were also decreased but still significantly differed from the control group (Figure 1 and Table 3).

Related to the effect on LH following treatment with Tri-Chromium picolinate, the mean serum LH level significantly increased in the induction group compared to the other groups. Furthermore, the average LH level in the HDTCP group was significantly lower than that in the LDTCP group. Similarly, the HDTCP group had a highly significant difference in comparison to the MDTCP group regarding these marker levels. Conversely, the HDTCP group had no significant difference when compared with the control group; HDTCP was similar to the control group, but LDTCP was increased to a very significant degree when compared with the control group. Additionally, the MDTCP group was significantly different than the control group. On the other hand, the HDTCP group did not significantly differ from the standard group,

Table 1. Significance of difference in testosterone hormone among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0.000	0.000	0.000	0.022	0.014
Induction			0.000	0.000	0.000	0.000
LDTCP				0.000	0.000	0.000
MDTCP					0.000	0.000
HDTCP						1.000
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

Table 2. Significance of difference in free testosterone hormone among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0.000	0.000	0.003	0.083	0.521
Induction			0.000	0	0	0
LDTCP				0.023	0.001	0
MDTCP					0.796	0.209
HDTCP						0.896
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

while both MDTCP and LDTCP groups were increased to a very significant degree in comparison with the standard group, as illustrated in Figure 1 and Table 4.

Concerning the effect on hormonal indicators after treatment with Tri-Chromium picolinate, the mean ratio of LH to FSH was significantly increased in the induction group compared to the other groups. The average LH/FSH ratio in the HDTCP group was significantly lower than that of the LDTCP group, while no significant difference was observed between the HDTCP and MDTCP groups regarding this ratio. Conversely, neither the MDTCP nor the HDTCP groups showed significant differences from the control group; both groups approached the control group in a dose-dependent manner. Additionally, both HDTCP and MDTCP groups were not significantly different than the standard group in regards to comparison to the standard group, as shown in Figure 1 and Table 5.

Regarding the effect of the treatment with Tri-Chromium picolinate on TSH, the average serum TSH level was increased to a very significant degree in the induction group in comparison to the other groups. The average serum TSH level in the HDTCP group was drastically reduced in comparison to the MDTCP and LDTCP groups. Meanwhile, the level of the marker in the treatment groups (HDTCP, MDTCP, and LDTCP) was significantly higher than in the control group, there was no significant difference between the groups HDTCP and the standard groups, as shown in Figure 1 and Table 6.

Table 3. Significance of difference in follicle stimulating hormone among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0.000	0.000	0.000	0.000	0.002
Induction			0.000	0.000	0.000	0.000
LDTCP				0.000	0.000	0.000
MDTCP					0.000	0.000
HDTCP						0.653
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

Table 4. Significance of difference in luteinizing hormone among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0	0	0.027	0.992	0.231
Induction			0	0	0	0
LDTCP				0	0	0
MDTCP					0.006	0
HDTCP						0.543
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

Table 5. Significance of difference in LH/FSH ratio among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0	0	0.989	1	1
Induction			0	0	0	0
LDTCP				0.002	0	0
MDTCP					0.976	0.959
HDTCP						1
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

Table 6. Significance of difference in thyroid stimulating hormone among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0.000	0.000	0.000	0.000	0.039
Induction			0.00	0.00	0.00	0.00
LDTCP				0.00	0.00	0.00
MDTCP					0.00	0.00
HDTCP						0.465
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

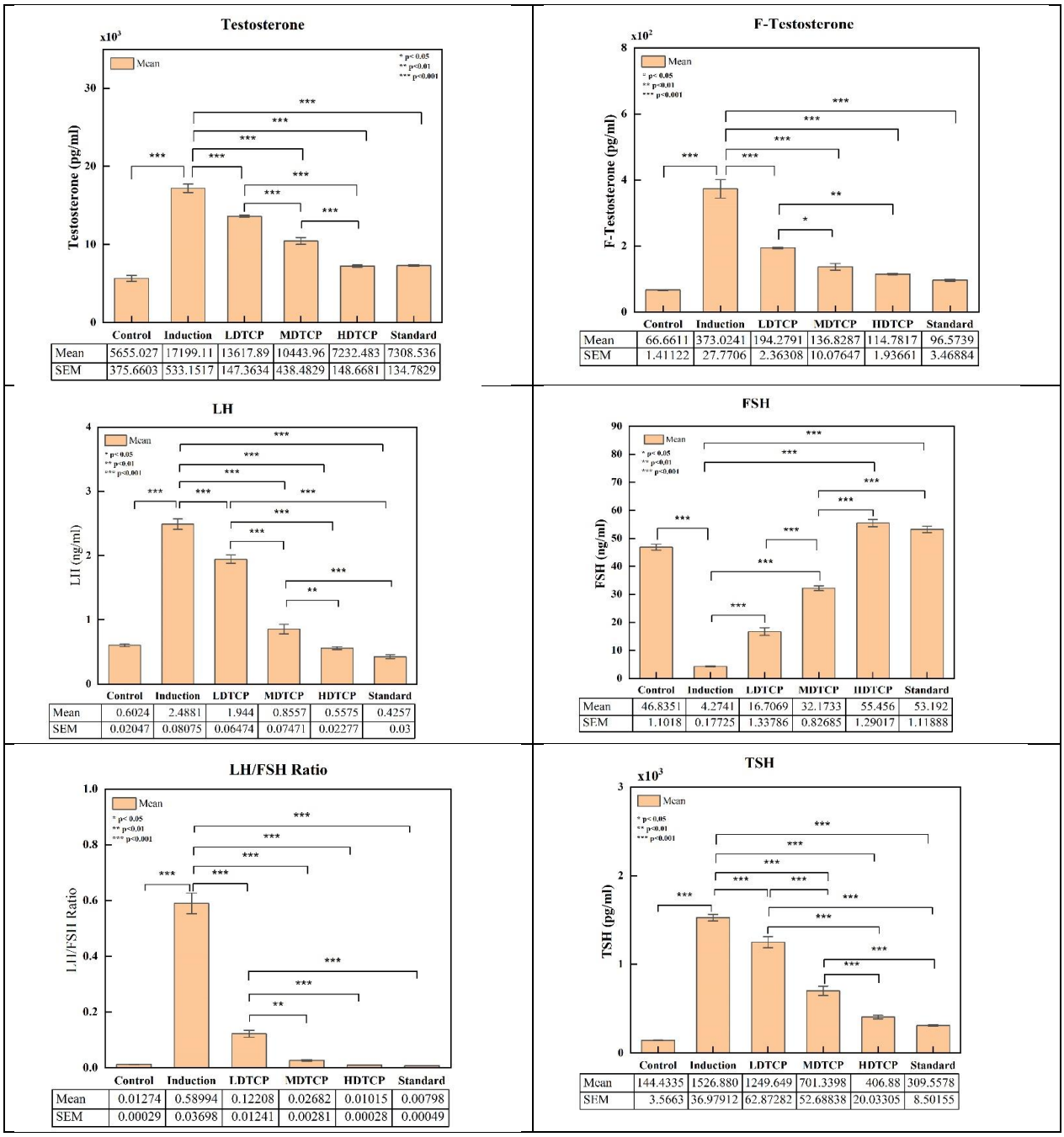


Figure 1. Evaluating the effect of different doses of Tri-Chromium picolinate on serum hormonal levels among studied groups. The data were represented by mean ± the standard error of the mean (SEM), * $P<0.05$ (significant difference), ** $P<0.01$ (highly significant difference), *** $P<0.001$ (very highly significant difference), TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, HDTCP: High dose TCP.

With respect to the effect on androgen synthetic pathway markers following treatment with Tri-Chromium picolinate, the mean serum androstenedione level was increased to a significant degree in the induction group in comparison to the other groups. The average serum androstenedione levels in the treatment groups (MDTCP, HDTCP and standard) were reduced to a significant degree when compared to the LDTCP group. However, there was no significant difference in the effect of the MDTCP group on these marker levels in comparison to the standard and control groups. Similarly, there was no significant difference in the effect of the HDTCP group on this marker level in comparison with the standard and control groups; the marker levels in both MDTCP and HDTCP groups were approximately equal to the control and standard groups (in a dose-dependent manner). Additionally, the average serum concentration of androstenedione in the MDTCP group was not significantly different than the HDTCP group, as can be seen in Figure 2 and Table 7.

In relation to the effect on AKR1C3, the average serum

Table 7. Significance of difference in androstenedione hormone among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0	0	0.696	0.355	0.865
Induction			0	0	0	0
LDTCP				0	0	0
MDTCP					0.993	1
HDTCP						0.948
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

Table 8. Significance of difference in AKR1C3 enzyme among the studied groups

Groups	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Control		0.000	0.000	0.002	0.054	0.024
Induction			0.001	0.000	0.000	0.000
LDTCP				0.351	0.000	0.000
MDTCP					0.000	0.000
HDTCP						0.999
Standard						

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

AKR1C3 demonstrated a highly significant increase in the induction group compared to the other groups, particularly when compared to the LDTCP group. The average serum level of AKR 1C3 in the HDTCP group was significantly lower than in the LDTCP and MDTCP groups. However, no significant difference was observed in the effect of the MDTCP group compared to the LDTCP group. Conversely, the marker level in the HDTCP group was significantly lower compared to the control group, as illustrated in Figure 2 and Table 8.

Effect of Tri-Chromium picolinate on the expression of CYP17A1 in ovaries rat tissue

Immunohistochemical evaluation

The CYP17A1 expression level was examined by immunohistochemical analysis. Figure 3 shows the cytoplasmic expression of this enzyme in the control, induction, LDTCP, MDTCP, HDTCP, and standard groups in ovarian rat tissues.

Immunohistochemical score evaluation

The expression of CYP17A1 in the current study was classified as negative (0), weak [I-4], moderate [5-8], and severe [9-12] based on the degree to which the reactions are intense and the frequency of cells that are stained in the different groups. The tissue's brownish color was considered an indication of a positive response (the binding of the antigen to the primary antibody). The results are categorized as scores from 0 to 12 based on the pathologist's evaluation, as illustrated in Table 9.

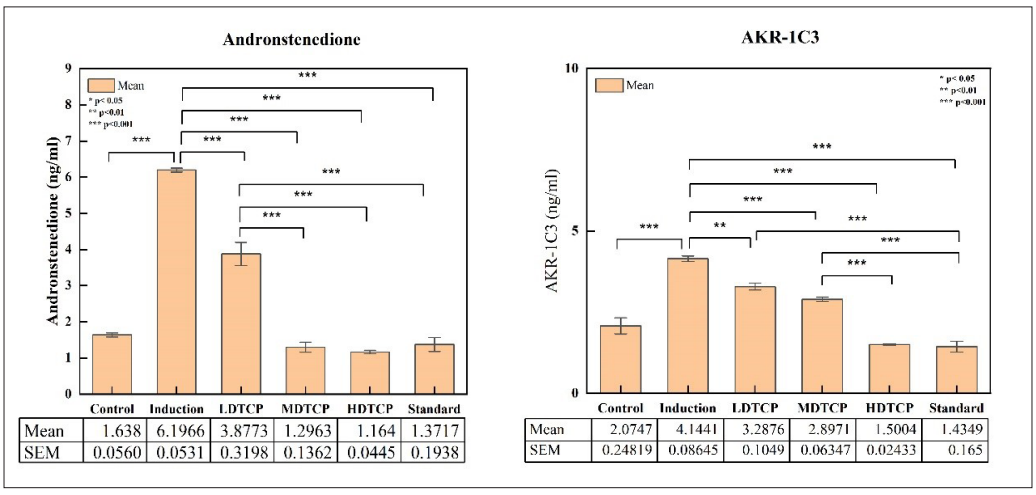


Figure 2. Evaluating the effect of different doses of Tri-Chromium picolinate on serum androstenedione levels and aldo-ketoreductase type 1C3 among studied groups. Data were represented by mean ± SEM (standard error of the mean), *** $P<0.001$(very highly significant difference), TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, HDTCP: High dose TCP.

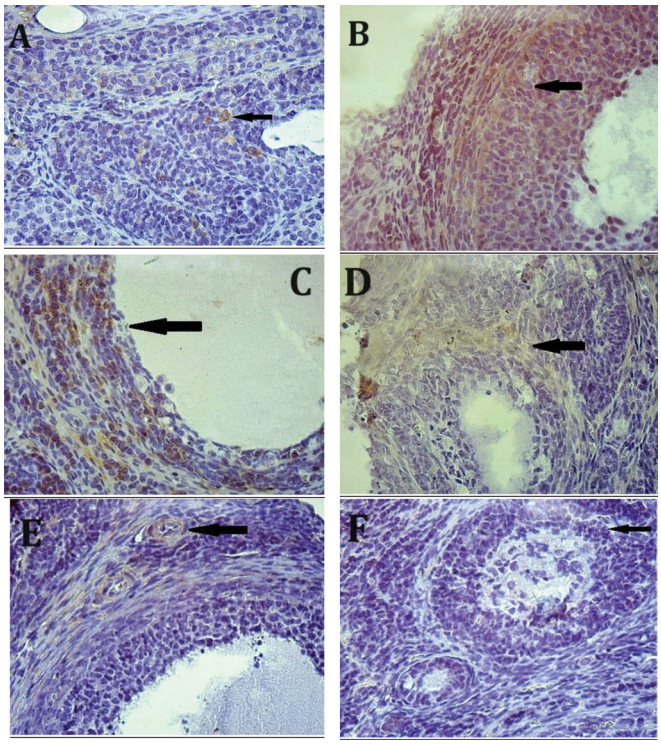


Figure 3. Immunohistochemical expression of CYP17A1 in the rat ovary A: the control group, B: the induction group, C: the LDTCP group, D: the MDTCP group, E: the HDTCP group, F: the standard group. The black arrow represents CYP17A1's expression. Magnification 100X, staining with hematoxylin.

Regarding the effect on CYP17A1, which is measured by IHC after treatment with Tri-Chromium picolinate, the mean level of CYP17A1 increased significantly in the induction group compared to the other groups. The average level of this enzyme in the MDTCP, HDTCP, and standard groups did not differ significantly from the control group. Conversely, the mean value of the LDTCP is significantly different than the control group. Ultimately, the average level of this enzyme in the HDTCP was not significantly different than the LDTCP, MDTCP, and standard groups. This is illustrated in Figure 4 and Table 10.

Table 9. Immunohistochemical score evaluation for CYP17A1

Groups	CYP17A1		
	Positive stained cells %score	Stain intensity score	Final score
Control	0	0	0
	1	2	2
	1	1	1
Induction	3	3	9
	4	3	12
	3	3	9
LDTCP	4	2	8
	2	3	6
	2	4	8
MDTCP	1	2	2
	2	3	6
	2	2	4
HDTCP	3	1	3
	2	1	1
	3	2	6
Standard	1	1	1
	2	3	6
	0	0	0

CYP17A1: cytochrome P45017A1, TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP. 0 → negative expression,1-4 → weak expression,5-8 → moderate expression, 9-12 → strong expression.

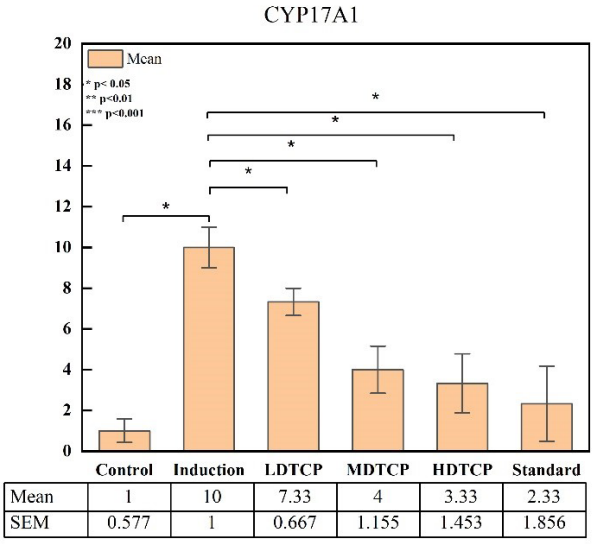
Discussion

There are a variety of drug treatments for PCOS; however, these are usually temporary, unhelpful, and often accompanied by many side effects (26). Currently, it is recommended that PCOS be treated with individualized, comprehensive treatments based on the patient's needs and complaints, the severity of metabolic disorders, and clinical symptoms. Many current PCOS treatments target only specific pathological processes and often cause side effects (27). Therefore, it is crucial to find a safe and effective drug that targets multiple signaling pathways to provide new ideas for the treatment of PCOS. As for the effects of different doses of Tri-Chromium picolinate treatment on hormone levels (testosterone, free testosterone, LH, FSH and TSH,), steroid hormones in follicular fluid play a vital role in follicular development, oocyte maturation, and ovulation (28). In the present study, testosterone enanthate significantly increased the mean serum levels of testosterone, free testosterone, luteinizing hormone, and the LH/FSH ratio in the induction group compared with the control group, and significantly decreased the mean serum level of FSH. These results were reported in

Table 10. Significance of difference in CYP17A1 enzyme among the studied groups

Groups	Induction	LDTCP	MDTCP	HDTCP	Standard
Control	0.046				
Induction		0.043			
LDTCP			0.072		
MDTCP				0.658	
HDTCP					0.5
Standard					

TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.



	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Mean	1	10	7.33	4	3.33	2.33
SEM	0.577	1	0.667	1.155	1.453	1.856

Figure 4. Evaluating the effects of different concentrations of Tri-Chromium picolinate on the expression of Cyp17A1 in the ovarian tissue of rats with PCOS. The data were represented by the mean ± the standard error of the mean (SEM), *$P<0.05$ (significant difference), TCP: Tri-Chromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, and HDTCP: High dose TCP.

a study by Wu et al (26). Untreated PCOS rats exhibited hyperandrogenism due to the inhibition of testosterone conversion to estradiol, resulting in elevated androgen levels and an increased LH: FSH ratio (27). Excessive LH levels stimulate ovarian endometrial cells to release large amounts of androgens and trigger apoptosis of follicular granulosa cells, leading to the occurrence of PCOS. In contrast, treatment with different doses of Tri-Chromium picolinate significantly normalized testosterone, free testosterone, FSH, LH hormone levels, and the LH/FSH ratio in a dose-dependent manner, as shown in Figure 1. These results are consistent with a previous study by Amr et al on the effects of chromium picolinate on free testosterone levels (29) and contradict a previous study by Tang X et al on the effects of chromium picolinate on LH and FSH hormones (30). As mentioned above, hyperglycemia or glucose intolerance can inhibit the production of sex hormone binding globulin synthesis in the liver which result in elevated serum levels of unbound steroid hormones. This may therefore affect the function of CYP17 A1, the main regulator of androgen biosynthesis in ovarian endometrial and stromal cells, leading to elevated androgen levels in women with PCOS (31). This may explain the reduction of these hormones after treatment with Tri-Chromium picolinate by improving insulin sensitivity. Considering the effects of Tri-Chromium picolinate on TSH, thyroid hormones play an important role in regulating metabolism and reproductive health. Thyroid hormone deficiency can affect reproductive function and fertility, delay puberty, and lead to irregular menstrual cycles (32). Subclinical hypothyroidism is associated with increased weight gain, increased sex hormone binding globulin, increased conversion of androstenedione to testosterone, and increased aromatization of estradiol. Barker et al reported that serum TSH levels were associated with dyslipidemia and a higher risk of major cardiovascular risk factors (33). In the present study, testosterone enanthate significantly increased mean TSH levels. This result is consistent with the previous findings of Cai et al, who demonstrated an association between hyperandrogenism and high TSH levels in women with PCOS (34). In contrast, treatment with different doses of Tri-Chromium picolinate significantly reduced TSH levels in a dose-dependent manner; these results suggest a link between insulin resistance and thyroid function, and Tri-Chromium picolinate may reduce TSH levels by improving insulin sensitivity (35), as shown in Figure 1. As for the effects of different doses of Tri-Chromium picolinate treatment on androgen synthesis pathways, in PCOS, insulin induces AKR1C3 in the adipocytes of PCOS patients, which serves as the main enzyme for the production of potent androgens (36). In the last two steps, it is converted into the synthesis of more active testosterone and dihydrotestosterone catalyzed by low-activity hormone precursors (such as androstenedione and androsterone), leading to ligand-dependent activation of the androgen receptor (AR). AR triggers fatty acid synthase (FASN), a key enzyme involved in lipogenesis (37). The induction of FASN has been shown to be dependent on AKR1C3 and AR. The subsequent lipid accumulation and increase in fat mass led to systemic insulin resistance and lipotoxic organ damage, and hyperinsulinemia furthermore enhances androgen production (38). In this study, androstenedione and AKR1C3 were significantly increased in the induced group compared with the control group, as shown in Figure 2. This result is consistent with the previous study of O'Reilly et al reported that insulin regulates AKR1C3 expression, thereby linking androgen excess and insulin resistance, a key metabolic feature of PCOS (38). In contrast, the levels of these markers were significantly reduced after administration of Tri-Chromium picolinate in a dose-dependent manner, as shown in Figure 2. Therefore, this supplement can reduce intracellular androgen production by inhibiting the activity AKR1C3. AKR1C3 inhibitors

competitively inhibit AKR1C3 and reduce AR signaling by enhancing AR degradation and ubiquitination. AKR1C3 is thought to play a dual role in PCOS: it catalyzes the production of potent androgens in adipocytes, leading to androgen excess, and triggers FASN by stabilizing AR in the absence of androgens. Therefore, AKR1C3 may serve as a therapeutic target for dual-function inhibitors to reduce cardiometabolic diseases in women with PCOS. Concerning the effect of different doses of Tri-Chromium picolinate treatment on CYP17A1 measured by IHC, disruption of the normal hypothalamic-pituitary-gonadal axis leads to increased testosterone and LH levels, resulting in the development of the disease state (39,40). Luteinizing hormone stimulates testosterone production in the theca layer by activating the phosphoinositide3-kinase/protein kinase B (PI3K/AKT) signaling pathway (41). LH-dependent Akt phosphorylation in the follicle is mediated by the PI3K signaling pathway, which stimulates the activity of 17-α-hydroxylase by increasing the expression of the ovarian enzyme CYP17A1 (41,42), a nuclear enzyme that regulates the conversion of steroidogenic progesterone to androgens, resulting in increased androgen levels. In the present study, testosterone enanthate significantly increased the expression of CYP17A1 compared with the control group, as shown in Table 9. This result is consistent with the previous findings of Marcondes et al, who reported that exposure to high testosterone levels in early life increased serum LH and testosterone levels, as well as LH/FSH ratio, ovarian theca interstitial area, and CYP17A1 gene expression in adult rats (43). In contrast, Tri-Chromium picolinate significantly decreased the expression of this enzyme in a dose-dependent manner, as shown in Table 9 and Figure 4. Therefore, the present study demonstrated the anti-androgenic properties of this supplement by completely blocking the PI3K/AKT signaling pathway and downregulating the CYP17A1 enzyme.

Conclusion

The results of this study show that Tri-Chromium picolinate significantly improves endocrine dysfunction by enhancing levels of testosterone, free testosterone, LH, FSH, and the LH/FSH ratio. Its effects on these hormone levels are comparable to those of the established anti-androgen drug cyproterone acetate. Furthermore, Tri-Chromium picolinate exhibits anti-androgenic activity by downregulating the expression of the CYP17A1 enzyme and decreasing the levels of AKR1C3 and androstenedione. The highest dose of Tri-Chromium picolinate (4 mg/kg) yielded the most favourable outcomes, comparable to those achieved with cyproterone acetate.

Limitations of the study

Several limitations of this study should be considered, including the short duration relative to human studies. Additionally, the small sample size of rats is a notable limitation. Furthermore, the induction of PCOS was on just the HA protocol and the need for other PCOS investigations.

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Conflicts of interest

The authors declare that they have no competing interests.

Ethical Issues

The research and the protocol of this study were in accordance with the guidelines of animal studies and were approved by the Ethics Committee of Mustansiriyah University of Pharmacy (Research no.19, Approval no.19 on 18/10/2023). Accordingly, we adhered to the guidelines for animal experiments as approved by the United States National Institutes of Health (NIH, 1978). Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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