Effects of Trichromium Picolinate on Histopathological Changes in Testosterone-Induced Polycystic Ovary Syndrome in Female Rats

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Abstract:

Polycystic ovary syndrome (PCOS) is the most prevalent disorder, establishing the single most common endocrine-metabolic disorder in women of reproductive age. Currently there are four recognized phenotypes of PCOS: 1) hyperandrogenism + oligo-anovulation + polycystic ovarian morphology; 2) hyperandrogenism + oligo-anovulation; 3) hyperandrogenism + polycystic ovarian morphology; and 4) oligo-anovulation + polycystic ovarian morphology, each having different long-term effects and metabolic consequences.

The histology of ovarian PCOS patients demonstrates that PCOS ovaries showed multiple ovarian cysts with a lack of corpus luteum, growing follicles, oocytes, granulosa, and theca cell layers. Chromium picolinate Chromium picolinate is a salt of the trace metallic element chromium (Cr). It is effectively treating hyperinsulinemia and hyperlipidemia.

This study aimed to investigate the effects of different doses of trichromium picolinate on the ovary histopathologic changes.

Methods: Forty-eight female albino rats were divided into six groups, with eight animals in each group. All groups were given testosterone enanthate 100 mg/kg/day by subcutaneous injection for 28 days, while the control group was given sesame oil 0.5 ml for 28 days. In the treatment stage, trichromium picolinate (1 mg, 2 mg, and 4 mg/kg/day) was given to groups III, IV, and V, respectively, for 42 days, and cyproterone acetate was given to group VI for comparison. At the same time, control and induction groups were treated with distilled water 0.5 mL orally for 42 days. In the current study, ovary histopathological changes were examined by hematoxylin and eosin staining.

Results: The control group showed apparently normal histology of ovarian rat's tissue with all types of follicles at different stages of maturation. Furthermore, the oocyte was intact, surrounded by granulosa and visible theca cell layers with numbers of corpus luteum. Conversely, the induction group confirmed PCOS in ovarian rat's tissue, which exhibited numerous cystic follicles and atretic

follicles, granulosa, and theca layer hyperplasia with a decrease in the number of developing follicles and the absence of corpus luteum. Histopathological examination of the treatment groups with different doses of trichromium picolinate demonstrated dose-dependent manner. Treatment with trichromium picolinate (1 mg/kg) mildly improved the histopathological changes of the ovary. When the dose was increased to 2 mg/kg, the score improved additionally, with the ovarian tissue resolving at 4 mg/kg of trichromium picolinate as in the cyproterone acetate group.

Conclusions: Trichromium picolinate can improve histopathological changes in the ovarian tissue of female rats.

Keywords: Polycystic ovary syndrome, Trichromium picolinate, testosterone enanthate, cyproterone acetate.

تاثير بيكولينات ثلاثي الكروميوم في التغيرات النسيجية المرضية لمتلازمة المبيض المتعدد الكيسات في إناث الجرذان المستحثة بهرمون التستوستيرون إينونثات

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خلاصة

متلازمة المبيض المتعدد الكيسات هي الاضطراب الأكثر انتشارًا، التي ينتج عنها اضطراب في الغدد والتمثيل الغذائي الأكثر شيوعًا لدى النساء في سن الإنجاب. يوجد حاليًا أربعة أنماط ظاهرية معترف بها لمتلازمة تكيس المبايض: 1) فرط الأندروجينية + قلة الإباضة + شكل متعدد الكيسات. 2) فرط الأندروجينية + قلة الإباضة. 3) فرط الأندروجينية + تشكل المبيض المتعدد الكيسات. و4) قلة الإباضة + تشكل المبيض المتعدد الكيسات، ولكل منها آثار صحية واستقلابية مختلفة على المدى الطويل. بيكولينات الكروم هو ملح من عنصر الكروم المعدني إنه يعالج بشكل فعال فرط أنسولين الدم وفرط شحميات الدم.

هدفت الدراسة الى متابعة تأثير جرعات مختلفة من بيكولينات ثلاثي الكروميوم على التغيرات النسجية المرضية للمبيض. المنهجية: تم تقسيم ثمانية وأربعين فأرًا ألبينو إلى ست مجموعات، كل مجموعة تحتوي على ثماني حيوانات. أعطيت جميع المجموعات هرمون التستوستيرون إينونثات 100 ملغ/كغ/يوم عن طريق الحقن تحت الجلد لمدة 28 يوما، في حين أعطيت مجموعة السيطرة زيت السمسم 0.5 مل لمدة 28 يوما. في مرحلة العلاج أعطيت بيكولينات ثلاثي الكروميوم 1 ملجم، 2 ملجم، 4 ملجم / كجم / يوم للمجموعات الثالثة والرابعة والخامسة على التوالي لمدة 42 يومًا وأعطيت أسيتات سيبروتيرون للمجموعة السادسة للمقارنة. في نفس الوقت عولجت مجموعات المراقبة والتحريض بـ 0.5 مل من الماء عن طريق الفم لمدة 42 يومًا. في الدراسة الحالية، تم فحص التغيرات النسيجية المرضية في المبيض عن طريق تلوين الهيماتوكسيلين والأيوسين. النتائج: أظهرت المجموعة الضابطة أنسجة طبيعية على ما يبدو لأنسجة الفئران المبيضية مع جميع أنواع الجريبات في مراحل مختلفة من النضج. علاوة على ذلك، تكون البويضة سليمة، ومحاطة بالحبيبات وطبقات خلايا القراب المرئية مع أعداد من الجسم الأصفر. على العكس من ذلك، أكدت المجموعة التحريضية وجود متلازمة تكيس المبايض في أنسجة الفئران المبيضية، والتي أظهرت العديد من الجريبات الكيسية والبصيلات الأذينية، والحبيبات، وتضخم طبقة القراب مع انخفاض في عدد الجريبات النامية وغياب الجسم الأصفر. أظهر الفحص النسيجي المرضي لمجموعات العلاج بجرعات مختلفة من بيكولينات ثلاثي الكروميوم طريقة تعتمد على الجرعة المعاملة ببيكولينات ثلاثي الكروم بجرعة (1 ملغم/كغم) أدت إلى تحسن طفيف في التغيرات النسيجية المرضية للمبيض. عند زيادة الجرعة إلى (2 ملجم / كجم)، تحسنت النتيجة أيضًا، حيث تم تحليل أنسجة المبيض عند (4 ملجم / كجم) من بيكولينات ثلاثي الكروم واظهرت نتيجة مقاربة كما في مجموعة خلات السيبروتيرون. الاستنتاجات: يمكن لبيكولينات ثلاثي الكروميوم تحسين التغيرات النسيجية المرضية على أنسجة المبيض في إناث الجرذان.

الكلمات المفتاحية: متلازمة المبيض المتعدد الكيسات،بيكولينات ثلاثي الكروم, التستوستيرون إينونثات ، اسيتات سيبروتيرون

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases in women of reproductive age[1]. It is associated with excess androgen levels, insulin resistance, and enlarged ovaries [2]. According to estimates, 1 in 10 women will experience PCOS before menopause. The precise etiology and pathophysiology of PCOS are still understood, despite the fact that the high luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio and increased frequency of gonadotropin-releasing hormone (GnRH) illustrate the hormonal disturbances of the disease [3]. Evidence suggests the role of several internal and external factors, which includes genetics, epigenetics, hyperandrogenism (HA), insulin resistance (IR), and environmental factors[4]. The steroidogenic acute regulating enzyme and the androgen-producing gene Cytochrome 450c17 are both increased by the interaction of LH and insulin. Also, IR increases Cytochrome p45017A1 (CYP17A1) activity, the enzyme responsible for producing androstenedione and testosterone [5]. Hyperinsulinemia, on the other hand, raises blood levels of free testosterone by decreasing hepatic sex hormone binding globulin (SHBG)[5,6]. Furthermore, the risk of other outcomes like cardiovascular diseases, type 2 diabetes mellitus, metabolic syndrome, depression, and anxiety is also linked to PCOS[7]. The normal histology of the ovary is defined by the presence of multiple follicles at varying stages of maturation. Primary follicles contain cuboidal granulosa cells in one or more layers, while secondary follicles are larger and have more granulosa cells, as well as internal and external thecal layers organized within the surrounding stromal tissue. In each cycle, just one follicle typically finishes the process, other follicles that had started to mature degenerate. Following ovulation and under the influence of luteinizing hormone, the ruptured follicle reorganizes into a specialized endocrine structure called the corpus luteum. The granulosa lutein cells secrete progesterone to maintain the uterus in a receptive phase and, if implantation occurs, continue to secrete progesterone during pregnancy in the presence of corpus luteum[8]. On the other hand, PCOS ovaries are characterized by increased ovarian size, the existence of multiple cystic follicles with luteinized theca layers, and a rise in the numbers of atretic follicles[9]. Trichromium picolinate is a salt of the trace metallic element chromium (Cr), it acts by decreasing insulin resistance by enhancing the insulin signaling pathway; chromium augments cellular glucose uptake. It has been shown to enhance the kinase activity of IR-β, to increase the activity of downstream effectors of insulin signaling phosphoinositide3-kinase /protein kinase B (PI3K/AKT) pathway and to enhance glucose transporter type 4 (Glut4) translocation to the cell surface. Chromium also down-regulates PTP-1B, the negative regulator of insulin signaling, and alleviates endoplasmic reticulum stress within the cells, rescuing IRS from JNK-mediated serine phosphorylation and subsequent ubiquitination. Transient up-regulation of AMPK by chromium leads to increased glucose uptake. Moreover, it mediates cholesterol efflux from the membranes causing Glut4 translocation and glucose uptake [10,11]. Chromium is considered one of 15 trace elements essential for proper physiological functioning of lipid and carbohydrate metabolism. Deficiency of chromium has been associated with a number of disorders, including symptoms of type 2 diabetes and cardiovascular disease[12]. The toxicity of this element is associated with the hexavalent form, which is 100 times more toxic when ingested compared to trivalent substances[13]. Animal studies have failed to show toxicity by histological or laboratory examination at doses of Cr as high as 15 mg/kg body weight[14]. The aim of the study was to evaluate the histopathological changes of the

ovaries after different doses of trichromium picolinate.

Materials and Methods:

Materials utilized in the presented study include trichromium picolinate and cyproterone acetate powder from TCI, Japan, testosterone enanthate ampule (250 mg/ml) from Panpharma, Jermany, xylazine vial (20 mg/ml) from Kepro, Holland, ketamine vial (10%) from Alfasan, Holland, solvent tween (80) from Research Product International RPI, USA, hematoxylin and eosin from Sigma, Jermany, and distilled water from pioneer, Iraq.

Animals

Fifty-four female albino rats were obtained from the local markets, their ages were 21 days, these animals were kept in a well-ventilated cage and had free access to water and food at a temperature of 25 ±2 °C in natural light /dark cycles and relative humidity of 55%. The rats were allowed to acclimatize for one week in the animal house /college of pharmacy before starting the experiment. The Mustansiriyah University College of Pharmacy's Ethical Community granted its permission according to the Ethical Committee on Animal Care (Research No.19 on 18 October 2023).

Study design

The animals were divided randomly into 6 groups each group contain eight rats. The study lasted for 70 days. A pilot study was carried out to confirm the PCOS induced by testosterone enanthate:

Group I (healthy control group): Eight female rats received only 0.5 ml of the sesame oil once daily by S.C for 28 days (induction period), and then 0.5 ml 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 1mg/100 gm 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 (G 18) for 42 days.

Group III (low dose of Trichromium picolinate): Eight female rats received daily doses of testosterone enanthate (1mg/100 gm B.W/day) as S.C for 28 days, then received trichromium picolinate dose (1mg/kg) once daily in D.W and tween (80) orally for 42 days.

Group IV (moderate dose of Trichromium picolinate): Eight female rats received daily doses of testosterone enanthate (1mg/100 gm B.W/day) by S.C for 28 days, then received trichromium picolinate dose (2 mg /kg) once daily in D.W and tween (80) orally for 42 days.

Group V (high dose of trichromium picolinate): Eight female rats received daily doses of testosterone enanthate (1mg/100 gm B.W/day) by S.C for 28 days, then received trichromium 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 (1mg/100 gm B.W/day) by S.C for 28 days, then received cyproterone acetate (2 mg /day) once daily in D.W and tween (80) orally for 42 days.

Preparation of drug and doses

The dose administration was performed at 8:30-9:30 AM daily to avoid hormonal fluctuation which may disturb the rat estrus cycle. Induction of PCOS was done by administration of testosterone enanthate at dose 1mg/100g B.W/day S.C at the dorsum of the neck for 28 days[15], it was prepared by diluting testosterone enanthate 250 mg/ml in 24 ml of sesame oil so that each 1 ml contained 10 mg of testosterone enanthate. Trichromium

picolinate doses was selected based on literature review[16,17], it was prepared by dissolving 15 mg of the pure drug (i.e. without any additives) in 15 ml distilled water and tween (80) so that each 1 ml contained 1 mg of the drug, it was given by oral gavage G 18 at doses 1mg, 2mg, and 4mg /kg/day for 42 days. Cyproterone acetate powder, it was used at dose 2mg /day[18] by oral gavage G 18 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. So that each 10 unit contained 1mg of cyproterone acetate.

Ovarian harvesting

The ovaries were located at the lower half of the abdominal cavity. Under euthanizing technique, the rats were fixed to the bench, then the rib cage was opened using a surgical pair of scissors. The ovaries were harvested and collected in formalin (10%) for histological examination to assess the effect of trichromium picolinate on ovarian tissues.

Histopathological study

Ovarian tissue processing and reading

The animals were sacrificed, and the ovaries were surgically removed. The tissue was processed as follows: fixation, dehydration and clearing, embedding, sectioning, staining, and slide reading [19,20].

Observational findings

A. Rat's body weight
   From day 21, animal body weight was measured on a weekly bases using a sensitive electronic balance.
B. Average ovary weight
   Animals were scarified, and the ovaries were taken and weighted to evaluate their average weight according to the following equation.

$$Average\ Ovary\ Weight = \frac{Wt.of\ Right\ Ovary + Wt.of\ left\ Ovary}{2}$$

Histological scoring system

Histological morphology changes such as primary follicles, secondary follicles, corpus luteum, atretic follicles, and cystic follicles were all examined and recorded by a pathologist, Prof. Dr. Israa Mahdi Al Saudany, and depending on previous studies[21].

Statistical analysis

Mean ± standard error of the mean (M±SEM) was used to represent the data. Statistical Packages for the Social Sciences (SPSS) version 25.0, was used for the statistical study. Analysis of variance (ANOVA) and a post hoc Tukey test were used to determine the significance of differential means. Significant, highly significant, very highly significant differences were defined as p-values lower than 0.05, 0.01, and 0.001, respectively.

Results

Regarding the effect on weight changes after treatment with trichromium picolinate, the mean value of rat's weight did not significantly differ in the induction group when compared with other groups. Likewise, the mean value levels in the treatment group HDTCP did not significantly differ when compared with the LDTCP, MDTCP, and standard groups, as shown in Figure (1). Considering the effect on average ovary weight after treatment with trichromium picolinate, the mean levels were increased to a very highly significant extent (p-value<0.001) in the induction group in comparison with the

control and standard groups and elevated significantly when compared with the LDTCP, MDTCP, and HDTCP. Meanwhile, there was no significant difference in the mean levels in the HDTCP group when compared with the LDTCP, MDTCP, and standard groups (p-value = 0.998,0.999 and 0.289, respectively), as shown in Figure (1).

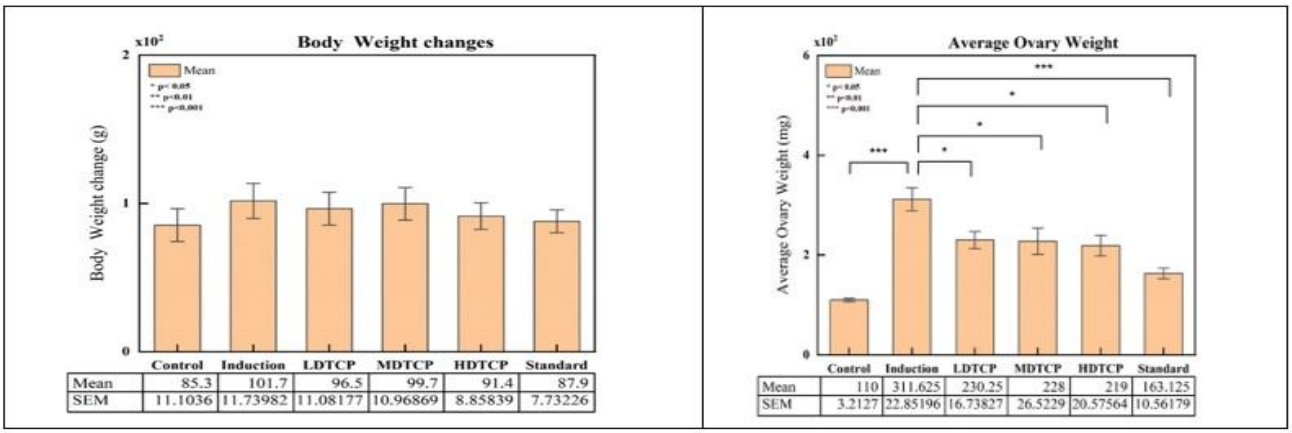

Figure (1): Evaluating the effect of different doses of trichromium picolinate on observational finding among studied groups. Data were represented by Mean ± SEM (standard error of mean), TCP: Trichromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, HDTCP: High dose TCP.

I. Morphology of the ovarian tissue

Light microscopic observation exhibited that the control group showed apparently normal histology of ovarian rat tissue with all types of follicles at different stages of maturation. Furthermore, the oocyte is intact, surrounded by granulosa and visible theca cell layers with numbers of corpus luteum, as shown in figure (2) a. Conversely, the induction group confirmed PCOS in ovarian rat's tissue, which exhibited numerous cystic follicles and atretic follicles, granulosa, and theca layer hyperplasia with a decrease in the number of developing follicles and the absence of corpus luteum, as shown in figure (2) b. Regarding the effect of LDTCP, this group showed ovarian rat tissue after LDTCP treatment with multiple cystic follicles and atretic follicles while the corpus luteum was formed, as shown in figure (2) c. However, the MDTCP group showed ovarian rat tissue after MDTCP treatment, with an increase in the number of primary and secondary follicles compared to LDTCP. Similarly, there was a decrease in the number of cystic follicles and atretic follicles compared to LDTCP and the formation of the corpus luteum, as shown in figure (2) d. Considering the effect of HDTCP and standard groups, these groups showed ovarian rat tissue after HDTCP and standard treatment. The ovarian cortex displayed the proliferation of numerous healthy follicles (primary and secondary follicles) and a decrease in the number of cystic follicles around one cyst. Furthermore, the numbers of atretic follicles were lower than those of LDTCP and MDTCP, with an increase in the numbers of corpus luteum; the morphology of ovarian tissue in these groups was approximately similar to that of the control, as shown in figures (2) e and f, respectively.

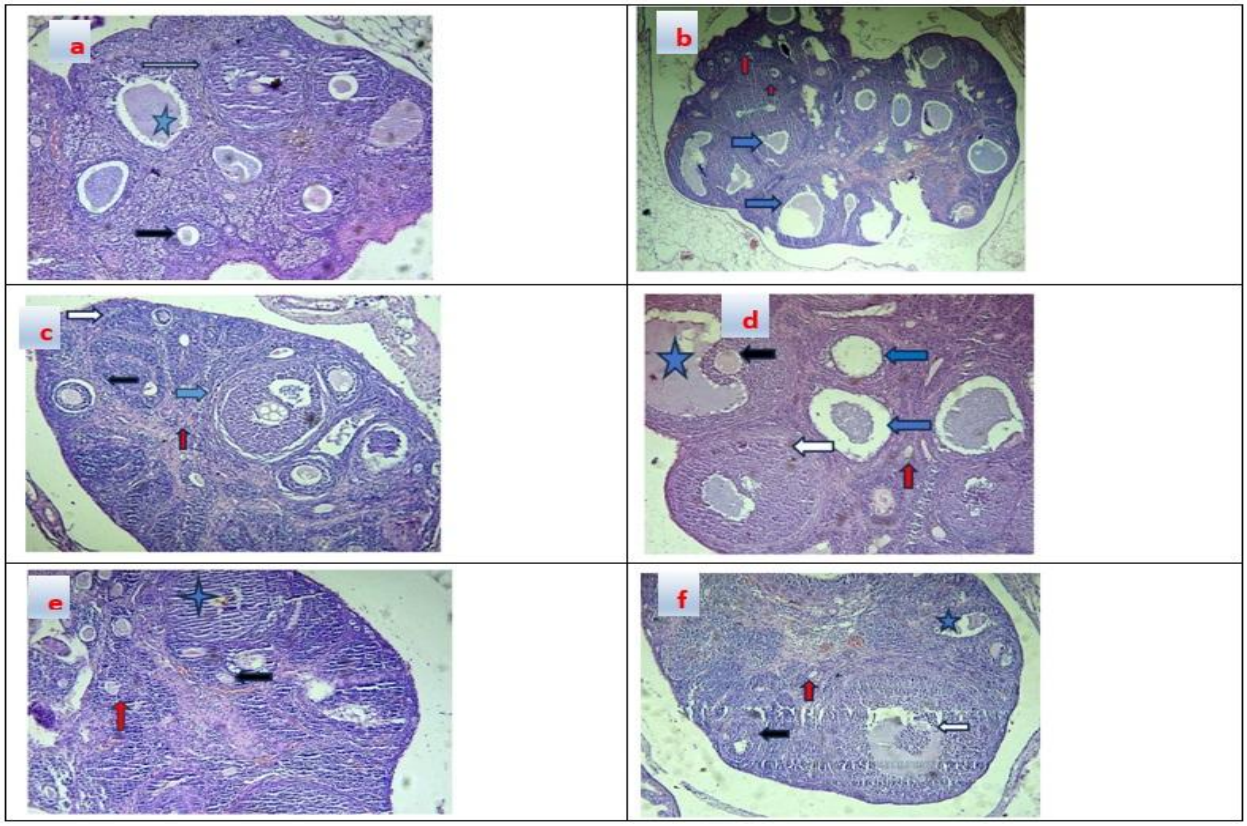

Figure (2): A light microscope section of rat ovaries. (a) control group showing that ovaries displayed apparently normal histology with several primary (black arrow) and secondary follicles (blue asterisk) and large numbers of corpus luteum (white arrow). (b) induction group showing that induction ovaries exhibited the formation of multiple cystic follicles (blue arrow) and atretic follicles (red arrow) with decrease in the numbers of primary and secondary follicles. (c) LDTCP group showing that LDTCP ovaries exhibited primary follicles with multiple cystic follicles and atretic follicles and formation corpus luteum. (d) MDTCP group showing that MDTCP ovaries exhibited numerous primary follicles and secondary follicles, with a decrease in the numbers of cystic follicles and atretic follicles and the formation of corpus luteum. (e) HDTCP group Showing that HDTCP ovaries displayed several developing follicles; primary follicles and secondary follicles, decreasing in the numbers of cystic follicles and atretic follicles with the formation of the corpus luteum. (f) Standard group showing that standard ovaries exhibited multiple primary follicles and secondary follicles, a decrease in the numbers of cystic follicles and atretic follicles and the formation of corpus luteum. Magnification:(A)100X, staining: Hematoxylin and Eosin.

II. Histopathologic scoring

The numbers of primary follicles, secondary follicles, corpus luteum, atretic follicles, and cystic follicles (per 4 µm section) in each group are illustrated in table (1).

Table (1): Histopathologic scoring in ovarian rat tissue among the studied groups

Criteria	Control	Induction	LDTCP	MDTCP	HDTCP	Standard
Primary follicle	7±0.577	3±0.577	5±0.577	6±0.577	6±0.577	7±0.577
Secondary follicle	5±0.577	3±0.577	3±0.577	4±0.577	4±0.577	5±0.577
Corpus luteum	4±0.577	0±0	1±0	1±0	2±0.577	3±0.577
Cystic follicle	0±0	5±0.577	5±0.577	2±0.577	1±0.333	1±0.333
Atretic follicle	0±0.577	7±0.577	6±0.577	5±0.577	4±0.577	3±0.577

LDTCP: Low dose trichromium picolinate, MDTCP: Medium dose trichromium picolinate, HDTCP: High dose trichromium picolinate.

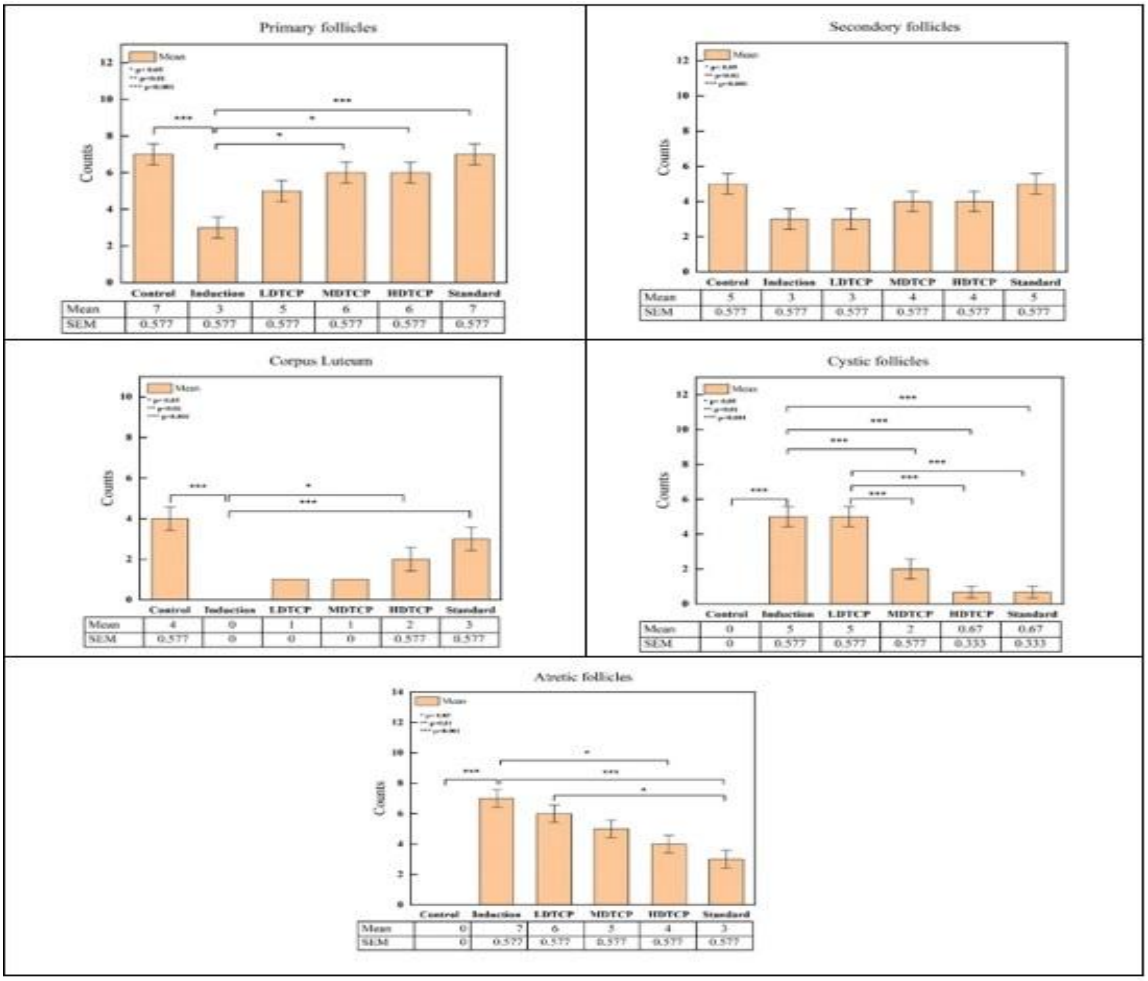

Figure (3): Evaluating the effect of different doses of trichromium picolinate on the number of primary follicles, secondary follicles, corpus luteum, cystic follicles, and atretic follicles in the ovarian tissue of PCOS-induced rats. Data were represented by Mean ± SEM (standard error of mean), *→*p*-value<0.05 (significant difference), *→*p*-value<0.001(very highly significant difference), TCP: Trichromium picolinate, LDTCP: Low dose TCP, MDTCP: Medium dose TCP, HDTCP: High dose TCP.**

Primary follicles (PF)

Regarding the effect on histopathologic changes after treatment with trichromium picolinate, the mean value levels of primary follicles in the induction group were decreased highly significantly when compared to the control group (p-value<0.004). The mean value levels of PF in the MDTCP and HDTCP increased significantly when compared with the induction group (p-value<0.05), while the mean value levels in the LDTCP group did not significantly differ when compared with the induction group (p-value = 0.816). Meanwhile, the marker levels in the treatment groups LDTCP, MDTCP, HDTCP, and standard did not significantly differ when compared with the control group (p-value = 0.214, 0.817, 0.817, and 1.0, respectively). Moreover, the mean value level in HDTCP did not significantly differ when compared with LDTCP, MDTCP, and standard groups (p-value 0.817, 1.0, and 0.817), as shown in figure (3).

Secondary follicles (SF)

Regarding the effect on histopathologic changes after treatment with trichromium picolinate, the mean value levels of secondary follicles in the induction group decreased but did not significantly differ when compared with other groups. Meanwhile, the mean value levels of SF in the treatment groups (LDTCP, MDTCP, HDTCP, and standard) elevated in a dose-dependent manner and did not significantly differ when compared with the control group. Finally, the mean value level of HDTCP did not significantly differ when compared with the LDTCP, MDTCP, and standard groups, as shown in figure (3).

Corpus luteum (CL)

Regarding the effect on histopathologic changes after treatment with trichromium picolinate, the mean value levels of corpus luteum in the induction group decreased to a very highly significant extent when compared to the control group (p-value<0.001). Meanwhile, the mean value levels of CL in the HDTCP elevated significantly when compared with the induction group (p-value< 0.042), while the mean value levels in the LDTCP and MDTCP also increased but did not significantly differ when compared with the induction group (p-value = 0.538). On the other hand, the mean value level of CL in the HDTCP group did not significantly differ when compared with the LDTCP, MDTCP, and standard groups (p-value = 0.538). Finally, the mean value level of the standard group did not significantly differ when compared with the control group (p-value = 0.538), as shown in figure (3).

Cystic follicles (CF)

Regarding the effect on histopathologic changes after treatment with trichromium picolinate, the mean value levels of cystic follicles in the induction group elevated to a very highly significant extent when compared to the control, HDTCP, and standard groups (p-value<0.001). Meanwhile, the mean value levels of CF in the LDTCP also decreased but did not significantly differ when compared with the induction group (p-value = 1.0). Moreover, the mean value level of CF in the MDTCP group declined to a highly significant extent when compared with the induction group (p-value<0.01). On the other hand, the mean value level of CF in the HDTCP group did not significantly differ when compared with the control, MDTCP, and standard groups (p-value = 0.894, 0.353, and 1.0, respectively), but was decreased to a very highly significant extent when compared to the LDTCP (p-value<0.001). Finally, the mean value level of the standard group did not significantly differ when compared with the control group (p-value = 0.894), as shown in figure (3).

Atretic follicles (AF)

Regarding the effect on histopathologic changes after treatment with trichromium picolinate, the mean value levels of atretic follicles in the induction group elevated to a very highly significant extent when compared to the control group (*p*-value<0.001). Meanwhile, the mean value levels of AF in the HDTCP decreased significantly when compared with the induction group (*p*-value<0.05), while both the LDTCP and MDTCP elevated to a very highly significant extent when compared to the control group (*p*-value<0.001) while, the mean value level of AF in the HDTCP elevated to a highly significant extent when compared with the control group (*p*-value<0.01). On the other hand, the mean value level of AF in the MDTCP and HDTCP groups did not significantly differ when compared with the standard group (*p*-value = 0.15 and 0.758, respectively), but the mean value level of AF elevated significantly when compared to the standard group (*p*-value<0.05). Finally, the mean value level of the standard group elevated significantly when compared with the control group (*p*-value<0.05), as shown in figure (3).

Discussion

Polycystic ovarian syndrome is one of the most common endocrine disorders among the women of reproductive age [22]. Defects in insulin action and hypothalamic-pituitary function are the causes of PCOS[23]. Evidences suggest the role of many factors include increased androgens and obesity, insulin resistance, type 2 diabetes, and oxidative stress [24,25]. The PCOS patients exhibit a reduced release of gonadotropin-releasing hormone together with increased secretion of LH in comparison to FSH [26]. Many current therapeutic approaches for PCOS exclusively target specific pathologic processes and frequently lead to adverse reaction [27]. Therefore, it is urgent to find a safe and effective drug acts on different pathway, so as to provide a new idea for the treatment of PCOS. One prominent hallmark of PCOS is elevated body weight and obesity. For example, in the United States, more than half of the patients with PCOS are either overweight or obese[28]. Obesity in women of reproductive age may result in dyslipidemia, irregular ovarian cycles, and anovulation, this imbalance results in mitochondrial malfunction, causing an increased production of reactive oxygen species (ROS), ultimately leading to ovarian damage and a higher incidence of follicular atresia [29]. Obesity has been shown to affect the phenotypic manifestation of PCOS and may be a major factor in the pathophysiology of chronic anovulation and hyperandrogenism. A number of abnormalities in the metabolism of sex steroids are linked to obesity, which raises androgen production and suppresses SHBG[30]. In the current study, the animal's weight was measured on a weekly basis for teen weeks during the induction and treatment period. The results demonstrate that PCOS-induced rats showed an increase in mean body weight as compared to the control group, as shown in Figure (1). Elevated testosterone levels can exacerbate abdominal obesity and inflammation, creating an unhealthy cycle in PCOS[31], this can explain the increase in body weight in PCOS rats. This result agrees with Wendy A. March *et al.* (2010), who reported that about 42% of PCOS patients have the complication of obesity[32]. On the other hand, TCP treatment groups did not exhibit any significant difference in mean body weight when compared with the induction group, this result agrees with a previous study done by Maleki V *et.al.* (2018), who reported that chromium supplementation does not improve weight loss in patients with polycystic ovary syndrome [33] and disagrees with a previous study done by Fazeilin S *et al.* (2017), who reported that using chromium

picolinate supplementation has beneficial effects on decreasing BMI [34]. Additionally, testosterone enanthate significantly elevated the average ovary weight in the PCOS group compared to the control group, as shown in Figure (1), and this result was in line with the result done by Samira Rajaei *et al*.[35]. The rise in body and ovarian weight could result from the accumulation of adipose tissue, the development of follicular cysts, and the storage of follicular fluid in the cystic follicles. Regarding the effect of different doses of trichromium picolinate treatment on the average ovary weight, the results showed that exposure to different doses of trichromium picolinate significantly decreased average ovary weight in PCOS rats in a dose-dependent manner, as shown in Figure (1), which may be responsible for decreasing fatty formation and decreasing follicular cysts (follicular fluid). In the current study, the histological examination of the PCOS ovaries revealed the existence of numerous fluid-filled subcapsular cystic follicles surrounded by a thin layer of degraded granulosa cells, with the absence of corpus luteum in comparison to the control group. These results agree with a previous study done by Ibrahim *et al.* (2022) who confirmed that PCOS ovaries showed multiple ovarian cysts with a lack of corpus luteum, growing follicles, oocytes, granulosa, and theca cell layers[36]. The decline in the number of corpora luteum can be linked to anovulation in PCOS, as the corpus luteum is crucial for producing the progesterone hormone, which regulates reproductive cycles and prepares the uterus for implantation in case of pregnancy [37]. Kafali *et. al.* (2004) reported that the anovulatory condition might be due to active FSH and LH levels and the reduced interplay between cells of ovaries, like theca cells and granulosa cells. Baravalle *et al*. (2006) reported a thin layer of granulosa cells, resulting in hyperplasia of theca cells[38]. In contrast, treatment of PCOS rats with different doses of trichromium picolinate improved PCOS criteria (primary follicles, secondary follicles, corpus luteum, cystic follicles, and atretic follicles) in a dose-dependent manner, as shown in table (1) and Figure (3). Treatment with trichromium picolinate (1 mg/kg) mildly improved the histopathological changes of the ovary. When the dose was increased to 2 mg/kg, the score improved additionally, with the ovarian tissue resolving at 4 mg/kg of trichromium picolinate as in the cyproterone acetate group; a high dose of trichromium picolinate significantly returned the ovarian tissue to some extent to normal architecture, with the appearance of follicles at various stages of development in the ovarian cortex, along with a significantly reduced number of cystic follicles and atretic follicles, suggesting restoration of the estrous cycle and normal functioning. As mentioned earlier, hyperinsulinemia raises blood levels of free testosterone by decreasing SHBG, so trichromium picolinate treatment in the present study reversed the increase in testosterone and LH levels by reducing insulin resistance, thereby reducing hyperandrogenism, a key feature of the development of PCOS. This could be explained by the ability of trichromium picolinate to improve the cystic follicles, antral follicles, and corpus luteum in PCOS rats. Several limitations of this study must be considered, such as the short study in comparison to a human study. Another limitation is the small number of rats. Other things are the fact that the induction of PCOS was on just the HA protocol, the lack of cytologic analysis, and the need for other PCOS investigations.

Conclusion and Recommendation

Results obtained from the current study revealed that trichromium picolinate improved the histological changes in ovarian

rats by increasing the number of healthy follicles and restoring the estrus cycle, represented by the formation of the corpus luteum. Many recommendations are considered like Comparing the effect of TCP as a dietary supplement to other supplements used currently in PCOS. Investigating other markers such as hormonal markers, oxidative markers, and cytology examination. Clinical studies are needed so that TCP can be utilized as a single or adjuvant therapy in combination with available therapeutic agents for treating PCOS.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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