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Original Article

Virgin coconut oil reverses behavioral phenotypes of letrozole-model of PCOS in Wistar rats via modulation of NRF2 upregulation

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الملخص

أهداف البحث: متلازمة المبيض المتعدد الكيسات هي اضطراب في الغدد الصماء يظهر مع مقاومة الأنسولين وفرط الأندروجين والعقم الفرعي. تم الإبلاغ عن أن زيت جوز الهند البكر له فوائد صحية، مثل الخصائص المضادة للالتهابات ومضادات الأكسدة المضادة للفيروسات. تبحث هذه الدراسة في تأثيرات النظام الغذائي الفموي التكميلي لزيت جوز الهند على الذاكرة والضعف الإدراكي في متلازمة المبيض المتعدد الكيسات التي يسببها دواء ليتروزول.

طريقة البحث: تم تقسيم ثلاثين من إناث الفئران بشكل عشوائي إلى خمس مجموعات. بصرف النظر عن المجموعة الضابطة، عولجت جميع الفئران الأخرى باستخدام ليتروزول لمدة 21 يوما للحث على متلازمة المبيض المتعدد الكيسات وعولجت بعد ذلك لمدة 14 يوما باستخدام 10 ٪ من زيت جوز الهند البكر وكلوميفين، وتم دمج الاثنين لعلاج المجموعة الأخيرة (زيت جوز الهند البكر + كلوميفين). تم إجراء ثلاثة اختبارات سلوكية عصبية هي "اختبارات الارتفاع بالإضافة إلى المتاهة، ومتاهة واي، واختبارات التعرف على الأشياء الجديدة".

النتائج: أظهرت نتائجنا ارتفاعا إحصائيا في تركيز الهرمونات الجنسية في مصل الدم في فئران متلازمة المبيض المتعدد الكيسات مقارنة بمجموعات التحكم والمجموعات المعالجة. أيضا، عكست جميع المجموعات المعالجة انخفاضا ملحوظا في تركيز المصل المنخفض من الكاتلاز والتعبير الجيني الخاضع للتنظيم لـ"إن آر اف-2" لعينات الحصين التي شوهدت في فئران متلازمة المبيض المتعدد الكيسات. بالإضافة إلى ذلك، أظهرت نتائجنا تعبيرا جينيا منظما لأستراز أستيل كولين في فئران متلازمة المبيض المتعدد الكيسات والتي تم إرجاعها بشكل ثابت بواسطة المجموعات المعالجة بزيت جوز الهند البكر.

الاستنتاجات: تم توضيح السلوك الشبيه بالقلق وضعف الذاكرة قصيرة المدى في هذه الدراسة. ومع ذلك، عكست مكملات زيت جوز الهند البكر كل هذا عن طريق تعديل التعبير الجيني "إن آر اف-2" و " إستيراز الأستيل كولين".

الكلمات المفتاحية: زيت جوز الهند البكر؛ متلازمة المبيض المتعدد الكيسات؛ الإجهاد التأكسدي؛ إستيراز الأستيل كولين؛ القلق؛ الذاكرة

Abstract

Objectives: Polycystic ovarian syndrome (PCOS) is an endocrine disorder associated with insulin resistance, hyperandrogenism, and sub-infertility. Virgin coconut oil (VCO) has been reported to have health benefits, such as anti-inflammatory, anti-oxidant, and antiviral properties. This study investigated the effects of dietary VCO supplementation on memory and cognitive impairment in female rats with letrozole induced PCOS.

Methods: Thirty female rats were randomly divided into five groups. All rats except controls were treated with letrozole for 21 days to induce PCOS and were subsequently treated for 14 days with 10% VCO, clomiphene (CLO), or VCO + CLO. Three neurobehavioral tests were conducted: elevated plus maze, Y maze, and novel object recognition tests.

Results: Our results indicated statistically elevated serum concentrations of sex hormones in rats with PCOS, compared with the control and treated groups. In addition, all treated groups showed significant reversal of the low serum concentrations of catalase and down-regulated gene expression of Nrf2 in the hippocampus seen in the PCOS rats. In addition, gene expression of acetylcholine esterase was up-regulated in PCOS rats, and was statistically reverted in the VCO treated groups.

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Conclusion: Anxiety-like behavior and impaired short-term memory were observed in PCOS rats; however, VCO supplementation reversed these effects by modulating the gene expression of Nrf2 and AchE.

Keywords: Acetylcholine esterase; Anxiety; Memory; Oxidative stress; Polycystic ovarian syndrome; Virgin coconut oil

Introduction

Polycystic ovarian syndrome (PCOS) is a heterogeneous clinical condition characterized by anovulation, metabolic imbalances, and endocrine and mental health abnormalities[1]. Globally, PCOS is responsible for approximately 10% of all cases of female infertility[2]; thus, clinical and pharmacological interventions are urgently required. Research is now focusing on delineating etiological pathways and possible drug targets for the treatment of PCOS.[3,4] According to current understanding, PCOS is associated with hypothalamic inflammation linked to a redox imbalance, thus resulting in hypothalamic−pituitary−gonadal axis dysregulation, central obesity, insulin resistance, hyperandrogenism, and infertility.[5–7] These underlying pathologies clinically manifest as cognitive and memory deficit, depression, anxiety disorder, and social phobia.[1,8–10]

Previous studies have reviewed the important role of the cholinergic system in memory and cognition.[62] Acetylcholine esterase (AchE) inhibitors have shown great potential in the management of patients with cognitive disorders, such as those in multiple sclerosis.[63]

Redox imbalance, which is indicated by excess formation of oxidants from reactive oxygen species (ROS) in the absence of an adequate anti-oxidant defense system, has been associated with the pathogenesis of PCOS (Turrens, 2003). The Kelch-like ECH-associated protein 1 (Keap1)/nuclear factor erythroid 2-associated factor 2 (Nrf2) pathway plays a central role in redox balance. Activation of Nrf2 results in the downstream expression of several anti-oxidant genes such as heme oxygenase, superoxide dismutase (SOD) and catalase (Suzuki and Yamamoto, 2017; Bellezza et al., 2018).

Virgin coconut oil (VCO), produced from coconuts, is rich in medium-chain fatty acids, polyphenols, and flavonoids. This functional food has broad health benefits, including anti-inflammatory, anti-oxidant, and antiviral properties, which have been well documented in the literature.[15]

The current study evaluated VCO, whose role as a redox modulator has been well documented,[2,3] in a **letrozole model of PCOS.** The results indicated that NRF2 gene upregulation was the mechanism underlying the reversal of behavioral phenotypes of PCOS in an experimental model.

Materials and Methods

Drugs

Clomiphene citrate tablets were purchased from Doppel Farmaceutici Sri Italy. Letrozole was purchased from Novartis Pharmaceuticals, India. SOD, prolactin, progesterone, estrogen, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH) detection kits were purchased from Fortress diagnostics, United Kingdom.

Animals

Thirty virgin female Wistar rats (190−210 g) were purchased from Ekiti State University, Ado Ekiti, Ekiti State, Nigeria, and housed in the animal laboratory of the Physiology Department, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria, at room temperature with a lighting schedule of 12 h light/12 h dark. Rats were fed rat chow and water *ad libitum*. This study was approved by the Ethics and Research Committee of the Faculty of Basic Medical Sciences, College of Medicine Ekiti State University, under protocol number EKSU/P100/2022/09/012, and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 2011).

Preparation of virgin coconut oil and supplemented diet

VCO was prepared according to a previously described method.[16] Briefly, fresh mature coconut was procured from a market in Ado-Ekiti, Ekiti State, Nigeria. The coconuts were grated, and their natural water was mixed and strained into a viscous paste until all creamy milk was released. The creamy milk was stored at room temperature for 48 h until adequate fermentation had occurred. Three layers were formed: a creamy mixture on top, VCO in the middle, and water on the bottom. The oil was gently collected and filtered into a container. The supplemented diet was subsequently prepared by weighing 10% w/w VCO from the freshly prepared oil, adding it to 90 g standard rat chow, and thoroughly mixing. The 10% VCO diets were mixed as needed.[17]

Experimental method

A total of 30 female rats were randomly divided into five groups (n = 6). Group 1 received 1 ml/kg of distilled water daily for 21 days. The remaining animals were administered 1 mg/kg letrozole daily for 21 days to induce PCOS[18] and were further divided randomly into four groups. Group 2 received oral doses of 1 mg/kg of vehicle. Group 3 received clomiphene citrate at 1 mg/kg in 0.9% NaCl.[19] Group 4 received a 10% VCO supplemented diet. Group 5 received clomiphene citrate and a 10% VCO supplemented diet. Except for group 1, all groups were treated for 15 days (from day 22−36).

Confirmation of PCOS with vaginal smear detection of the estrous cycle

Vaginal smears were performed for estrous cycle determination. The estrous cycle phase was studied through daily observations of vaginal smears of rats between 7 am and 9 am for 21 days, as previously reported (Kausar*et al*., 2021) Microscopic inspections of the vaginal smears of rats with polycystic ovarian cysts demonstrated a diestrous phase with a predominant leukocyte cell type, thus confirming the anovulatory status of the animals.

Behavioral tests

All tests were conducted in the physiology experimental room on experimental day 35 (elevated plus maze and Y maze) and day 36 (novel object recognition) within the hours of 7:00 pm to 11:00 pm daily.

Elevated plus maze test

The animals were tested for anxiety with an elevated plus maze[20] on experimental day 35. The maze was constructed from black plywood. The four arms were elevated 80 cm above the plane surface and consisted of two opposing open arms (45 × 10 cm) and two opposing closed arms with similar dimensions (50 cm wall height). On the test day, each rat was placed at the central platform of the maze facing the open arm and was allowed to explore the arms for 5 min. A blinded observer recorded the time spent in either the open or closed arm in seconds, as well as the number of entries into the arms. The percentage time spent in open arms was calculated as the total time spent in an open arm/300 s × 100. The percentage entry was calculated as (total open arm entry/total number of entries into both arms) × 100, and was also applicable to the closed arms. The anxiety index was calculated as 1 − [(time spent in open arms/total time spent) + (open arm entries/total entries)]/2. Entry was assumed when the four limbs of a rat were completely inside the particular arm.

Y maze

The animals' short-term working memory was evaluated with the Y maze apparatus.[21] A smooth plywood structure comprised three arms (25 × 10 × 75 cm) with 120° between them.

On the experimental day, each rat was placed at the center of the maze and allowed to explore the three arms for exactly 6 min. A blinded observer recorded the pattern of arm entries, which were initially designated A, B, and C. Normally, rats are expected to visit a relatively new arm and not return to the arm that they just came from or a recently visited arm. Thus, a rat with a higher visit sequence of arms A, B, and C consecutively without repetition is considered to show spontaneous alternation performance (SAP) indicating better short-term memory performance. The %SAP was calculated as (number of SAP)/(total number of arm entries − 2) × 100. Entry was assumed when the four limbs of a rat were completely inside a particular arm. Between tests, the maze was cleaned with ethanol (20%) soaked cotton wool, then allowed to dry properly before use for the next rodent.

Novel object recognition tests

Cognitive performance was examined with the novel object recognition task experimental approach.[22] This procedure has three separate phases. First was the habituation phase, in which each rat was allowed to freely explore an empty test arena (70 × 25 × 50 cm) for 10 min on experimental days 34 and 35. Next was the familiarization phase, wherein the rat was placed in the test chamber, in which two familiar objects (red toy of similar size and shape) were placed in two adjacent corners. The rat was allowed to explore the objects for 10 min and then was returned to its cage. The last phase, performed 1 h after the prior phase, included new similar toys that had the same shape, size, and color (novel objects) but were different from the two previous familiar objects (in shape and color), and were placed at adjacent corners of the chamber. Thus, a total of four objects were placed in the chamber: two similar familiar objects and two similar novel objects. Each rat was allowed to explore freely for 5 min. The time spent exploring each object was monitored and recorded with a camera placed on the roof of the chamber. The recognition ratio was calculated as the time spent exploring the novel object divided by the total time spent exploring both objects.

Between tests, the chamber was cleaned with ethanol (20%) soaked cotton wool, then allowed to dry properly before use for the next rodent, to eliminate olfactory memory.

Biochemical analysis

Blood samples were collected from the experimental rats through retro-orbital puncture 24 h after the last treatment dose. The blood samples were stored in universal bottles, separated by centrifugation at 3000 rpm/min for 15 min, and frozen at 20 °C until hormonal assays of GnRH, FSH, LH, testosterone, estrogen, and SOD, according to the manufacturer's instructions.

Gene expression in brain tissue

According to the manufacturer's protocol, for each group, the brain (hippocampus) was harvested in TRIzol reagent (Thermo Fisher Scientific) and was used for total RNA isolation. The extracted RNA was subjected to DNase I treatment (Thermo Fisher Scientific). DNA-free RNA was then transcribed into cDNA with a ProtoScript® First Strand cDNA Synthesis Kit (NEB). PCR amplification was performed with OneTaq® 2X Master Mix (NEB)[23] with forward and reverse primer sets for Nrf2, nuclear factor-kappa B (NFKB), and AchE.

ENE	Forward primer	Reverse primer
NRF2	GTCAGCTACTC CCAGGTTGC	CAGGGCAAG CGACTGAAATG
AchE	ACGTGAGCCT GAACCTGAAG	CTCGTCCAGC GTGTCTGTG

Statistical analysis

Bar graphs were plotted to reflect mean ± SEM (n = 6) values of the gene/β-actin ratio of the gel (1.5% agarose in TAE buffer) image for each sample, as computed in ImageJ. The photographs are representative snapshots of pooled samples. For statistical analysis, GraphPad Prism version 9 was used to perform one-way analysis of variance (ANOVA) followed by *post hoc* Tukey tests. The statistical significance threshold was set at $p < 0.05$.

Results

Figure 1 indicated statistically elevated serum concentrations of GnRH, FSH, and LH in the PCOS rats compared with the control and treated groups, thus

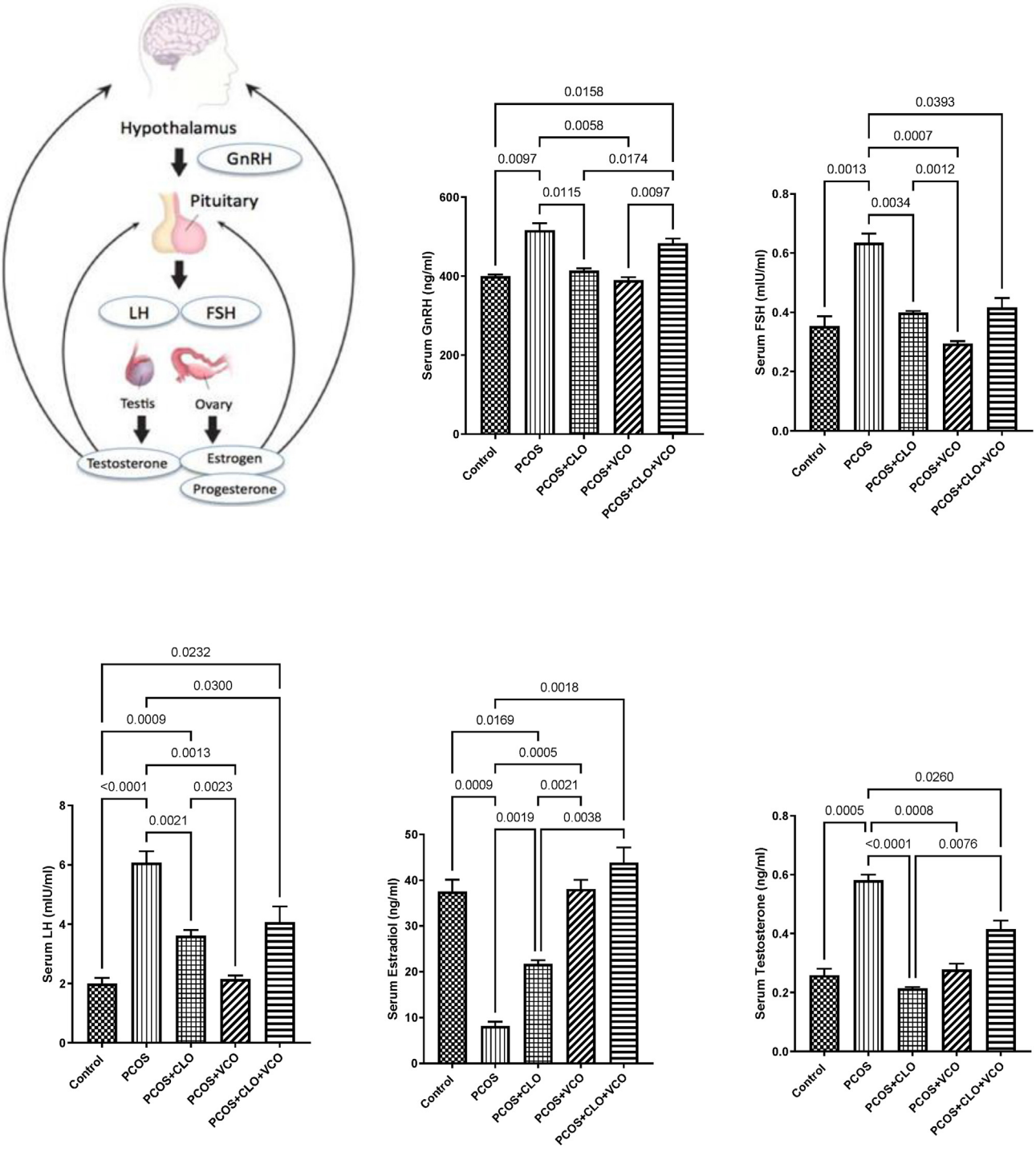


Figure 1: Hypothalamic-Pituitary-Gonadal Axis. Data are expressed are means ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. $p < 0.05$. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); GnRH (gonadotropin releasing hormone); FSH (follicle stimulating hormone); LH (luteinizing hormone)

reflecting the physiological negative feedback response to significantly low serum levels of estradiol in the PCOS rats. Compared with rats receiving standard drug treatment, rats treated with only VCO showed significantly lower levels of anterior pituitary hormones ($p = 0.0487$ and 0.0285, respectively) and statistical reversion of serum estradiol and testosterone levels ($p = 0.0002$ and < 0.0001, respectively). Animals receiving combined treatment showed no significant improvements over VCO only treated rats.

Hypothalamic-Pituitary-Gonadal Axis.[64]

Figure 1 data are expressed are means ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. $p < 0.05$. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); GnRH (gonadotropin releasing hormone); FSH (follicle stimulating hormone); LH (luteinizing hormone).

Our results in Figure 2A and B indicated that all treated groups showed significant reversal of the low serum concentrations of catalase and SOD observed in the PCOS group. However, the group receiving combined treatment showed a statistically higher concentration than the other two treated groups ($p = 0.0075$ and 0.0119, respectively) in Figure 2A. The down-regulated gene expression of Nrf-2 in PCOS rats, as shown in Figure 1B, was also statistically reversed in the VCO, CLO, and CLO + VCO treated groups.

Figure 2 (A and B): Serum concentrations of catalase and SOD, respectively, expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); SOD (superoxide dismutase). **C:** Brain (hippocampus) homogenate anti-oxidant gene expression in the control, PCOS, and treatment groups. Values of quantified bands from each sample for the indicated genes across the five groups are expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. The gel image is representative of the pooled samples. (Each bar graph represents control normalized relative expression (specific gene/β-actin). PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); NFR-2 (nuclear factor-erythroid related factor 2).

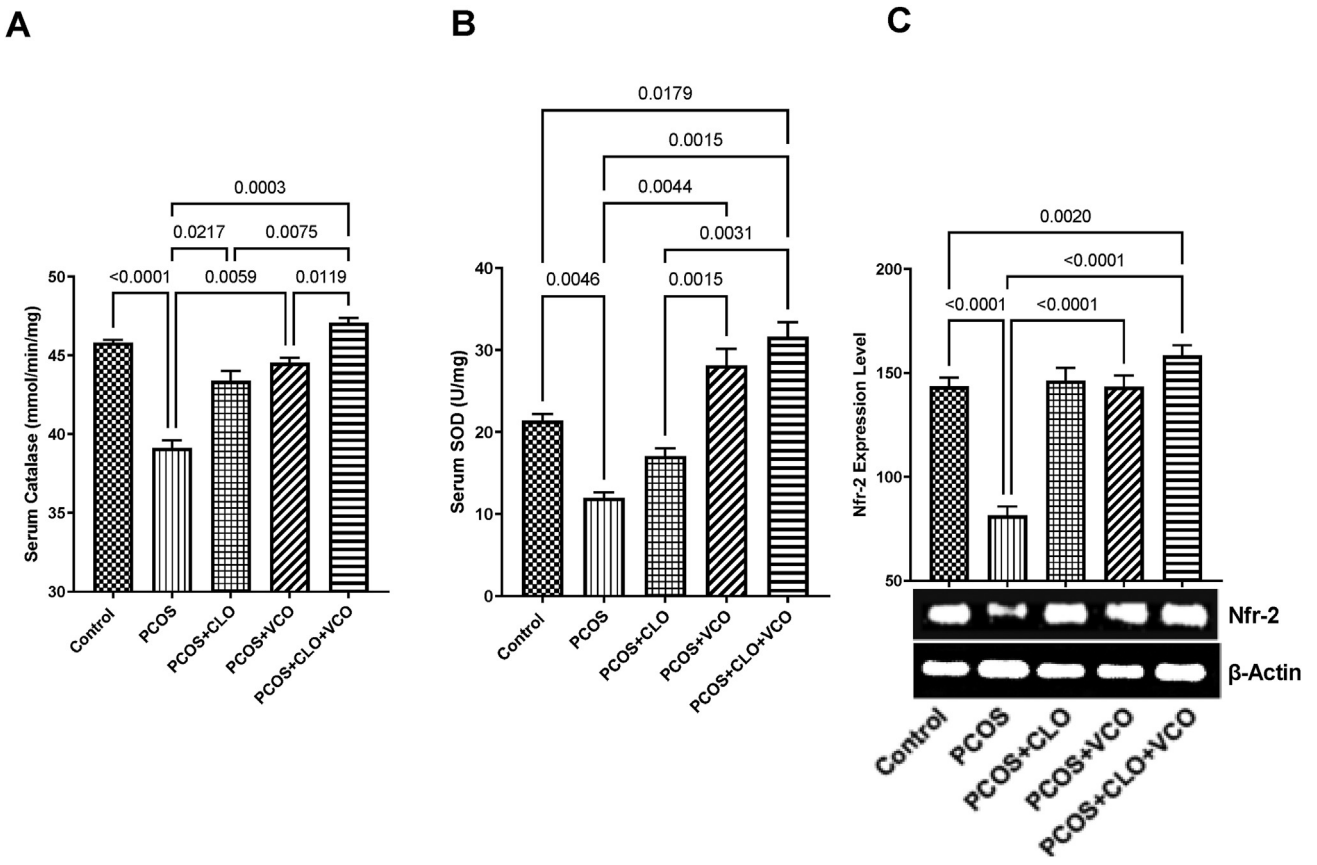


Figure 2: Anti-oxidant properties of VCO in rats with letrozole induced PCOS. (A and B): Serum concentrations of catalase and SOD, respectively, expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); SOD (superoxide dismutase). C: Brain (hippocampus) homogenate anti-oxidant gene expression in the control, PCOS, and treatment groups. Values of quantified bands from each sample for the indicated genes across the five groups are expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. The gel image is representative of the pooled samples. (Each bar graph represents control normalized relative expression (specific gene/β-actin). PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); NFR-2 (nuclear factor-erythroid related factor 2)

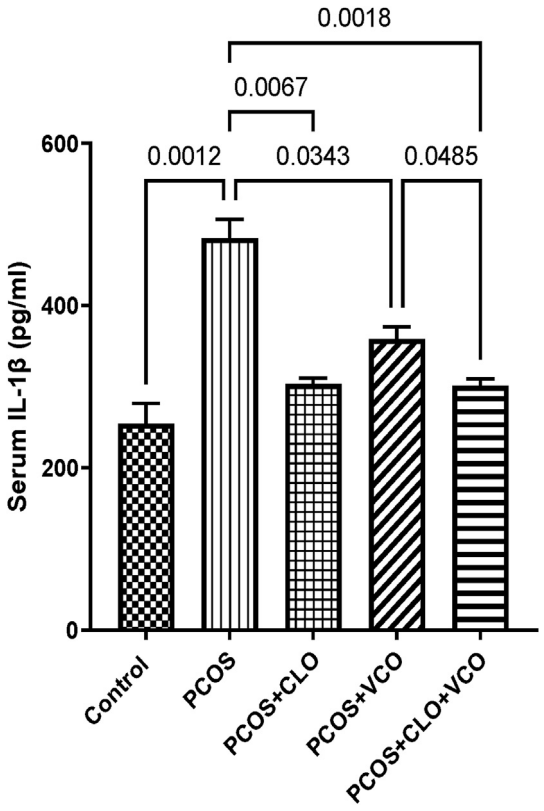


Figure 3: All treatment groups demonstrate relatively potent anti-inflammatory properties in rats with letrozole induced PCOS. Serum concentration of IL-1B, expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); IL-1β (interleukin 1-beta)

The high serum levels of IL-1B in PCOS rats, as shown in Figure 3, were statistically reversed in VCO, CLO, and CLO + VCO treated animals.

Figure 3 Serum concentration of IL-1B, expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); IL-1β (interleukin 1-beta).

The PCOS rats showed a statistically evident anxiety-like behavior, which was reversed in the VCO only and VCO + CLO treatment groups (Table 1). Statistically, PCOS rats spent little time in the open arm and the most time in the closed arm, as compared with the VCO and CLO + VCO groups ($p = 0.0005$ and <0.0001) ($p = 0.0017$ and <0.0001), respectively. The standard drug showed the same trend in the diseased rats. The PCOS + CLO + VCO rats showed statistically greater cognitive stability than the VCO only treated rats ($p = 0.0379$).

As shown in Table 2, rats treated with CLO and VCO only spent a significantly higher proportion of time exploring the novel object than did rats in the PCOS group and the group receiving combined treatment ($p = <0.0001$, <0.0001, <0.0001, and <0.0001, respectively). Similar trends were observed in the calculated recognition ratio results. SAP tests (Figure 4A and B) showed that the CLO and VCO only treatments statistically reversed the short-term memory deficits observed in the diseased group and combined treatment group.

Figure 4 effects of VCO in short-term memory cognitive functional tests and Y-maze assessment tests in female rats with letrozole induced PCOS. Data are expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. PCOS (polycystic ovarian syndrome); CLO

Table 1: Behavioral effects on rats with letrozole induced PCOS in the elevated plus maze test.

	Control	PCOS	PCOS + CLO	PCOS + VCO	PCOS + CLO + VCO
%Time (Open)	55.17 ± 2.7	9.33 ± 1.5[a]	8.33 ± 0.9[a]	25.2 ± 2.0[abc]	39.83 ± 3.3[abcd]
%Time (Closed)	43.0 ± 3.4	90.5 ± 1.6[a]	91 ± 1.3[a]	75 ± 2.0[abc]	60.17 ± 3.3[abcd]
%Open Arm Entry	67.7 ± 5.0	12.5 ± 5.59[a]	11.83 ± 7.5[a]	47.2 ± 3.83[bc]	57.5 ± 4.0[bc]
%Closed Arm Entry	32.2 ± 4.9	88 ± 5.4[a]	88.3 ± 7.8[a]	52.7 ± 3.7[bc]	42.5 ± 4.0[bc]
Anxiety Index	0.38 ± 0.03	0.9 ± 0.02[a]	0.9 ± 0.03[a]	0.64 ± 0.02[abc]	0.52 ± 0.02[abcd]

Data expressed are means ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. a, b, c, d: $p < 0.05$ vs control, PCOS, PCOS + CLO, PCOS + VCO, and PCOS + CLO + VCO, respectively. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); % (percentage).

Table 2: Behavioral effects in rats with letrozole induced PCOS in novel object recognition tests.

	Control	PCOS	PCOS + CLO	PCOS + VCO	PCOS + CLO + VCO
Ex. Familiar (Sec)	107 ± 4.8	154.5 ± 2.7[a]	95.2 ± 2.8[b]	91.7 ± 3.3[b]	160 ± 4.5[acd]
Ex. Novel (Sec)	154.2 ± 3.8	113.2 ± 2.8[a]	170 ± 4.6[b]	169.7 ± 4.1[b]	95.2 ± 7.1[acd]
Rec Ratio	0.59 ± 0.01	0.42 ± 0.01[a]	0.61 ± 0.01[b]	0.64 ± 0.01[b]	0.37 ± 0.02[acd]

Data expressed are means ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. a, b, c, d: $p < 0.05$ vs control, PCOS, PCOS + CLO, PCOS + VCO, and PCOS + CLO + VCO, respectively. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); Ex (Exploration time); Rec Ratio (recognition ratio).

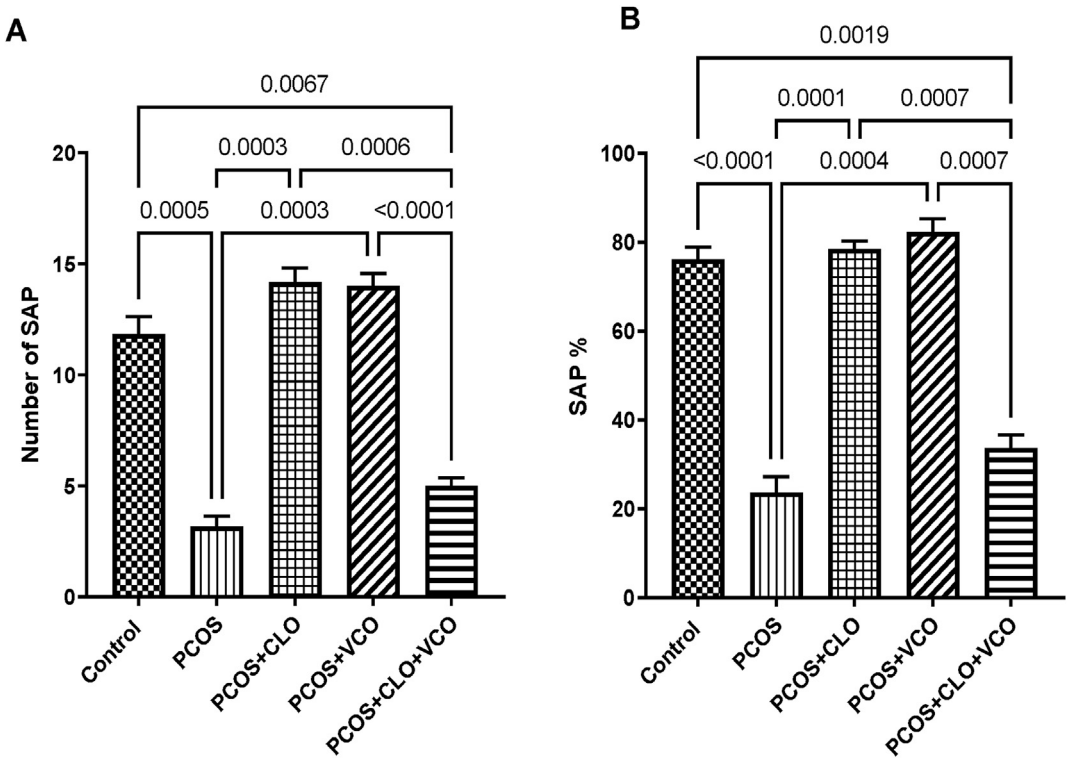
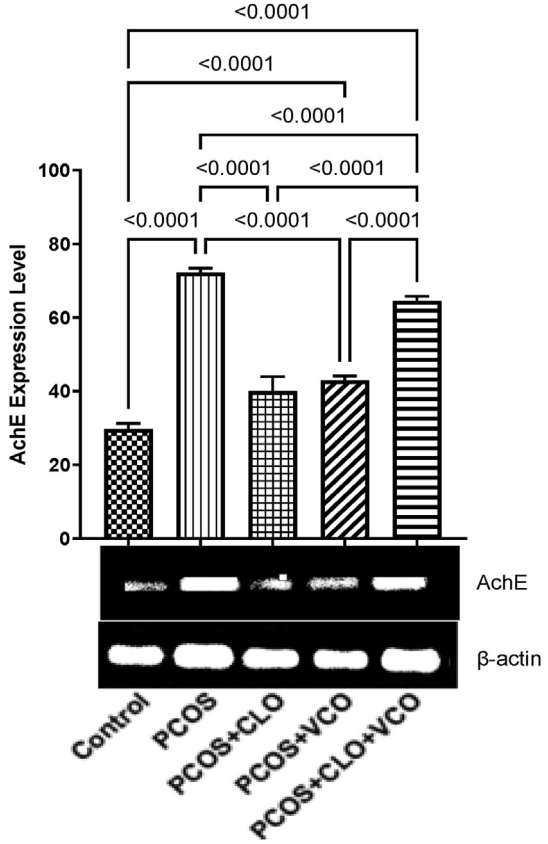


Figure 4: VCO reverses anxiety-like behavior in rats with letrozole induced PCOS compared with clomiphene treated animals. Effects of VCO in short-term memory cognitive functional tests and Y-maze assessment tests in female rats with letrozole induced PCOS. Data are expressed as mean ± SEM, n = 6. Data were analyzed with one-way way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); SAP (spontaneous alternation performance) SAP% (spontaneous alternation performance ratio)



(clomiphene); VCO (virgin coconut oil); SAP (spontaneous alternation performance) SAP% (spontaneous alternation performance ratio).

Figure 5 VCO and CLO reverse the up-regulation of acetylcholine esterase observed in rats with letrozole induced PCOS

Our results indicated up-regulated gene expression of AchE in PCOS rats (Figure 5). This effect was statistically reverted in the VCO and CLO and CLO + VCO treated groups ($p \leq 0.0001$, <0.0001, and <0.0001, respectively). No synergistic effect of CLO and VCO was observed, because single doses resulted in lower mRNA expression of AchE than that observed in the combined treatment rats.

Figure 5: VCO showed an optimizing effect on the short-term memory deficits seen in rats with letrozole induced PCOS. Brain (hippocampus) homogenate gene expression in the control, PCOS, and treatment groups. Values of quantified bands from each sample for the indicated inflammatory genes across the five groups are expressed as mean ± SEM, n = 6. Data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. The gel image is representative of the pooled samples. (Each bar graph represents control normalized relative expression (specific gene/β-actin). PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); AchE (acetylcholine esterase)

Figure 5 Brain (hippocampus) homogenate gene expression in the control, PCOS, and treatment groups. Values of quantified bands from each sample for the indicated inflammatory genes across the five groups are expressed as mean ± SEM, n = 6. Data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test. The gel image is representative of the pooled samples. (Each bar graph represents control normalized relative expression (specific gene/β-actin). PCOS (polycystic ovarian syndrome); CLO (clomiphene); VCO (virgin coconut oil); AchE (acetylcholine esterase).

Discussion

The treatment of PCOS has been classified into (1) nonpharmacological treatments (such as dietary modification and exercise) that cause a relative decrease in body weight, insulin resistance, and generation of oxidative and pro-inflammatory cytokines, thereby indirectly diminishing the neurological manifestations of PCOS, and (2) pharmacological treatments, which are often used to supplement nonpharmacological treatments.[24,25]

This experiment was designed to investigate the effects of VCO as am oral dietary supplement on memory and cognitive impairment in female rats with letrozole induced PCOS.

Our diagnosis of PCOS was based on biochemical evidence of sex hormonal dysfunction and hyperandrogenism,[26,27] in agreement with the Rotterdam criteria of 2003 (Rotterdam, 2003).[28] Our results showed that letrozole-only treated animals had significantly elevated serum GnRH, LH, and testosterone, and low FSH values. These effects were ameliorated by VCO treatment, which restored the hypothalamic–pituitary–gonadal axis, a physiological endocrine pathway that is essential in the pathogenesis of PCOS.[29] Of note, VCO-only treated rats showed better treatment efficacy than clomiphene-only treated rats. In contrast, the combined treatment group showed the statistically lowest efficacy, as compared with the other treatment groups.

Strong links exist among obesity, redox oxygen imbalances, chronic low-grade inflammation,[30] and disruption in neural pathways in the hypothalamus. These pathways involve Kisspeptin-neurokinin B and dynorphin neurons (KNDγ). Kisspeptin-neurokinin B, when activated, increases GnRH pulsatility, whereas activation of dynorphin neurons inhibits this effect.[31] Moreover, the melanocortin system, which drives the energy balance at the cellular level,[32] comprises the leptin-pro-opiomelanocortin (POMC)-melanocyte-stimulating hormone-melanocortin 3/4 (MC3/4R) receptor in the hypothalamus, producing anorectic behavior after eating,[33,34] and insulin activation of the orexigenic Agouti-related protein (AgRP) expression inhibits gene expression of POMC, thereby increasing food intake behavior.[35]

Generalized chronic low-grade inflammation leads to activation of several inflammatory pathways locally in the hypothalamus that result in potentiation of AgRP neurons and leptin resistance,[36] thereby increasing insulin resistance,[37,38] endoplasmic reticulum stress, and mitochondrial dysfunction and regeneration defects[39,40] in brain neurons and microglia.[37] In addition, poor hypothalamic–pituitary–gonadal axis control, poor hypothalamic glucose homeostasis control, and diminished memory and cognitive function[41,42] are observed in PCOS.[43,44]

The overt generation of free radical oxygen activates Nrf2, thus leading to the generation and release of antioxidants (catalase and SOD), which are used by the body to combat ROS generation via NRSF/ARE mechanisms.[23] Our work indicated that oral VCO supplementation potentiated this pathway, thus resulting in statistically higher Nrf2 gene expression in the frontal brain lobe, and higher catalase and SOD serum levels than those in the PCOS rats. Likewise, VCO demonstrated good anti-inflammatory properties, as previously reported,[45] by decreasing the serum concentration of IL-1β below those in diseased animals. This efficacy was similar between the control drug and the combined treatment.

Beyond its role in glucose homeostasis, insulin has been identified to play crucial roles in cognition,[46] learning, and memory, by promoting neural and microglial growth[47] and the release of neurotransmitters including AchE,[46,48] and supporting hippocampal synaptic plasticity.[49] Thus, the insulin resistance associated with PCOS tends to negatively affect cognition. In addition, we postulated that the brain neural oxidative and chronic inflammation status in PCOS accompanies memory impairment and cognitive dysfunction. Our results indicated that VCO treated animals showed greater mood stability than clomiphene treated and PCOS rats as they explored the open arm. Notably, a synergistic response was observed in animals receiving combined treatment.

Likewise, VCO and clomiphene restored the short-term memory impairment in the letrozole-only treated rats. Nevertheless, no synergistic effect was observed between VCO and clomiphene, as noted in the case of the anxiety response. In contrast, clomiphene (a selective estrogen receptor modulator) has been found to ameliorate memory impairment.[50,51] One possible mechanism is modulation of the AchE enzyme, which has not previously been reported. The clomiphene and VCO treated animals had statistically lower gene expression of AchE than the diseased and combined treated animals. In line with our results, several findings have implicated a defective brain cholinergic system in the pathophysiology of dementia and Alzheimer's disease.[52,53] Islam et al., 2017[54] have also reported that the memory and learning impairments seen in older people and patients with Alzheimer's disease are closely associated with low acetylcholine and elevated AchE activity at the synapses. This neurological finding is strongly associated with a high oxidative stress state in the brain cells (neurons and supporting cells, such as glia cells and astrocytes).[55]

The antioxidant and anti-inflammatory effects of VCO are well supported by the scientific literature[56,57]; consequently, VCO has been considered as a routine daily food supplement.[58,59] Furthermore, its phytochemical properties include those of P-coumaric and ferulic phenolic compounds, which have been documented to have potent anti-oxidant and anti-inflammatory effects.[60,61] VCO also contains flavonoids and polyphenols, which are good free radical scavengers that might have contributed to the neuroprotective effects of VCO observed in this study. Ultimately, this study indicated the upstream mopping up of generated free radicals and attenuation of the chronic low-grade inflammation by VCO, which directly or indirectly resulted in PCOS and its various clinical reproductive

and non-reproductive manifestations, such as impaired short-term memory and impaired cognitive function.

Conclusion

This study demonstrated anxiety-like behavior and impaired short-term memory via redox oxygen imbalance and chronic low-grade inflammation, which up-regulated AchE gene expression in the brain. However, oral VCO supplementation ameliorated the hormonal imbalance and neurotoxic manifestations of PCOS by modulating Nrf2 and AchE gene expression.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Ethical approval was obtained from the Ekiti State University (reference number EKSU/P100/2022/09/012) with a date of approval of November 16, 2021.

Authors' contributions

OOA and AJA were responsible for the conceptualization of this study and the acquisition of relevant data. All aspects of the study were undertaken, carefully examined, reviewed, and approved by both authors. They are both responsible for the content of the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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