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Effect of nanocurcumin on fertility in murine model of polycystic ovary syndrome

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Abstract

The precise pathophysiology of polycystic ovary syndrome (PCOS) is not well-founded. In an attempt to fill this gap, the current study was executed to probe the effect of nanocurcumin (NCC) on ovarian tissue, *in vitro* fertilization (IVF) and pre-implantation embryo development in a mouse model of PCOS. Fifty adult female mice were randomly categorized into five equal groups including non-treated control and PCOS (receiving 0.20 mg estradiol valerate (EV) intra-peritoneally once a day for 21 days) as well as $NCC_{12.50}$ + PCOS, NCC_{25} + PCOS and NCC_{50} + PCOS (receiving respectively 12.50, 25.00 and 50.00 mg kg^{-1} NCC daily along with EV injection through oral gavages for 21 days) groups. Subsequently, ovarian histo-architecture and total anti-oxidant capacity, and malonaldehyde and catalase levels as well as *in vitro* fertilizing potential, early embryonic development and serum testosterone concentration were analyzed. Results showed that NCC in a dose-dependent manner improved ovarian cyto-architectural organization and oxidant/anti-oxidant balance along with IVF rate and pre-implantation embryo development in PCOS mice. These findings revealed that NCC at the doses of 25.00 and 50.00 mg kg^{-1} could alleviate PCOS-linked reproductive disruptions in female mice.

Introduction

Polycystic ovary syndrome (PCOS) as a heterogeneous endocrine disorder affects approximately every 1 in 10 women worldwide.[1] Although the exact pathophysiology of PCOS has not been fully understood, several external and internal factors including environmental, nutritional and genetic factors, physical and emotional stresses, epigenetic alterations, insulin resistance, hyper-androgenism, inflammatory and oxidative reactions and obesity have been reported to play pivotal etiological roles in the pathogenesis of this abnormality.[2] As a multi-factorial disorder, PCOS is characterized by anovulation, dysfunctional and cystic ovaries, high androgen levels and menstrual irregularities.[3] Besides, it has been widely recorded that PCOS is associated with several untoward complications such as metabolic syndrome, cardiovascular diseases, type 2 diabetes mellitus and depression.[4]

Unfortunately, there is not any United States Food and Drug Administration approved medication specifically for PCOS so far.[5] From this point of view, more attentions are needed to find novel beneficial medications to be considered as therapeutic options in PCOS management strategies.

Curcumin (CC; diferuloylmethane), a polyphenol component of turmeric (*Curcuma longa*), is a well-accepted anti-oxidant compound being reported to possess anti-bacterial, anti-carcinogenic, anti-diabetic, anti-fungal, cholesterol-lowering, anti-inflammatory and anti-viral activities.[6] Accordingly, it has been shown that CC has protective effects against premature ovarian failure in mice[7] and ovarian damages induced by ischemia-reperfusion in rats.[8] Furthermore, the estrogenic properties of CC have been demonstrated formerly.[9] Recently, clear evidence suggests CC encapsulation into nanoformulations (nanocurcumin [NCC]) to promote its biological functions leading to the increased bioavailability and solubility as well as slow metabolization.[10]

In support of this fact, it has been indicated lately that NCC as an anti-inflammatory nutraceutical is able to modulate the inflammation/autophagy in the metabolic complications of PCOS.[11]

In light of this concept, the aim of this study was to look into the effects of NCC on ovarian histological structure, *in vitro* fertilization (IVF) and pre-implantation embryo development in a mouse model of estradiol valerate (EV)-induced PCOS.

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Materials and Methods

Animals and treatment. Fifty adult healthy female mice (age: 8 weeks and body weight: 25.00 - 30.00 g) were provided from Animal Resource Center, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. The animals were group-housed in a temperature-controlled room (20.00 ± 2.00 ˚C) under the relative humidity of 40.00 ± 5.00% and 12-hr darkness/light cycle. Tap water and food were provided *ad libitum* throughout the experiment. The mice were allowed to become accustomed to the experimental conditions for 2 weeks and then, they were randomly divided into five equal groups (n = 10) including non-treated control and PCOS, receiving 0.20 mg EV (Aburaihan Pharmaceutical Co., Tehran, Iran) intra-peritoneally (IP) once a day for 21 days,[12] as well as $NCC_{12.50}$ + PCOS, NCC_{25} + PCOS and NCC_{50} + PCOS groups, receiving respectively 12.50, 25.00 and 50.00 mg kg^{-1} NCC (ExirNanoSina Co., Tehran, Iran) daily along with EV injection through oral gavages for 21 days.[13] In order to confirm PCOS induction, vaginal smears were collected daily and examined microscopically (Olympus, Tokyo, Japan) using Giemsa stain.[2] All experimental procedures were approved by the Institutional Research Ethics Committee under the Ethical Code of IR.IAU. URMIA.REC.1401.102.

Sampling. Twenty-four hr after the last administration, five mice from each experimental group were euthanized following general anesthesia induced by IP injection of 90.00 mg kg^{-1} ketamine (Alfasan, Woerden, The Netherlands) and 10.00 mg kg^{-1} xylazine (Alfasan)[14] and blood samples were immediately collected for serological analyses. The left ovaries were immediately harvested and stored at – 80.00 ˚C for biochemical analyses and the right ones were trimmed free of fat and undergone fixation in 10.00% neutral buffered formalin for histological studies.

Hormonal assay. Serum concentrations of testosterone were determined using rat/mouse enzyme-linked immuno-sorbent assay kit (Cosmo Bio Co., Tokyo, Japan) based on the manufacturer's instructions and expressed as ng mL^{-1}.

Oxidant/anit-oxidant status markers determination. Malondialdehyde (MDA), catalase (CAT) and total anti-oxidant capacity (TAC) levels in homogenized ovarian tissues were measured according to Tappel and Zalkin,[15] Aebi[16] and Katalinic *et al.*[17] methods, respectively. The values were expressed as µmol per g tissue.

Ovarian histological examination. The fixed right ovaries were embedded in paraffin, serially sectioned at 5.00 µm thickness, mounted on glass slides, stained with hematoxylin and eosin and examined using light microscopy (Olympus). The numbers of atretic antral and cystic follicles as well as corpora lutea were computed considering the criteria described previously.[7,12] An oocyte surrounded by multiple layers of cuboidal granulosa cells containing one or more antral spaces, possibly with a cumulus oophorus and thecal layer was considered as an antral follicle. Atretic follicles were follicles entering degenerative processes (oocyte nucleus shrinkage, chromosomes and cytoplasm dissolution, granulosa layer reduction and follicular membrane cells hypertrophy) without ovulation.[7]

Oocyte collection, IVF and pre-implantation embryo development monitoring. The 14 hr after super-ovulation stimulation based on the method reported formerly,[18] female mice (n = 5) were sacrificed following general anesthesia induction (see above) and their oviducts were immediately excised (Fig. 1A) and placed in Petri dishes containing human tubal fluid (HTF; Sigma, St. Louis, USA) medium. Under stereo zoom microscope (Model TL2; Olympus), cumulus-oocyte complexes were found (Fig. 1B) and moved to the fertilization droplets under mineral oil-containing HTF medium. After that, the capacitated caudal epididymal sperms (1.00×10^6 mL^{-1} HTF) collected from a mature healthy male mouse (age: 10 weeks and body weight: 27.90 g) were introduced to the medium. Fertilization rates were then calculated following 4 - 6 hr incubation at 37.00 ˚C under 5.00% CO_2. Then, zygotes were transferred into the fresh medium and cultured for 5 days to assess the percentages of two-cell embryos, morulae and blastocysts (Figs. 1C - 1F).[19]

Statistics. The variables were analyzed by one-way analysis of variance followed by Tukey multiple range *post hoc* analyses using SPSS Software (version 22.0; IBM Corp., Armonk, USA). The Shapiro-Wilk and Levine tests were used to examine the normality of data distribution and variances homogeneity, respectively. The data were expressed as the mean ± standard error and a probability < 5.00% was considered significant.

Results

Biochemical findings. As represented in Table 1, PCOS caused marked elevations in the levels of testosterone and MDA respectively in serum and ovarian tissue compared to the control group. While, tissue levels of TAC and CAT reduced significantly in PCOS group compared to the control one. Interestingly, NCC administration at the doses of 25.00 and 50.00 mg kg^{-1} significantly decreased serum concentration of testosterone and increased ovarian tissue levels of TAC and CAT compared to the non-treated PCOS group.

Histological findings. In comparison with the control group, EV-induced PCOS notably increased the number of cystic and atretic antral follicles and reduced the number of corpora lutea. Remarkably, NCC administration at the doses of 25.00 and 50.00 mg kg^{-1} following PCOS induction ameliorated ovarian histological features as evidenced by reduced number of cystic and atretic antral follicles and higher numbers of corpora lutea (Fig. 2).

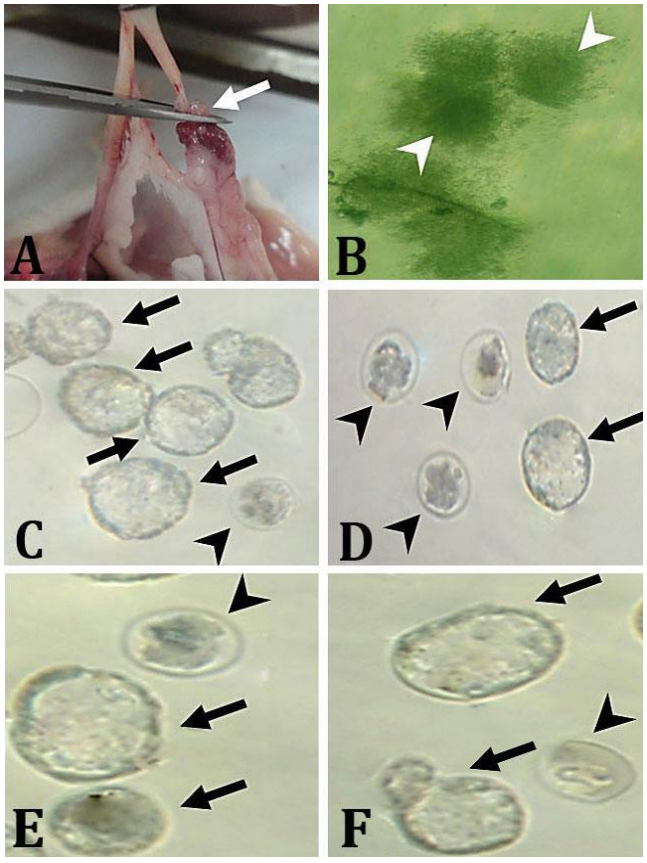

Fig. 1. Oocyte collection and *in vitro* pre-implantation embryo development. **A)** To collect the oocytes, the swollen oviductal ampullary portion (white arrow) was found; **B)** Using stereo zoom microscope, cumulus-oocyte complexes (white arrowheads) were picked up. Differentiation to blastocysts (black arrows) as well as arrested embryos (black arrowheads) can be observed in **C)** control, **D)** PCOS, **E)** NCC_{25} + PCOS, and **F)** NCC_{50} + PCOS groups (×200). PCOS: Polycystic ovary syndrome; NCC: Nanocurcumin.

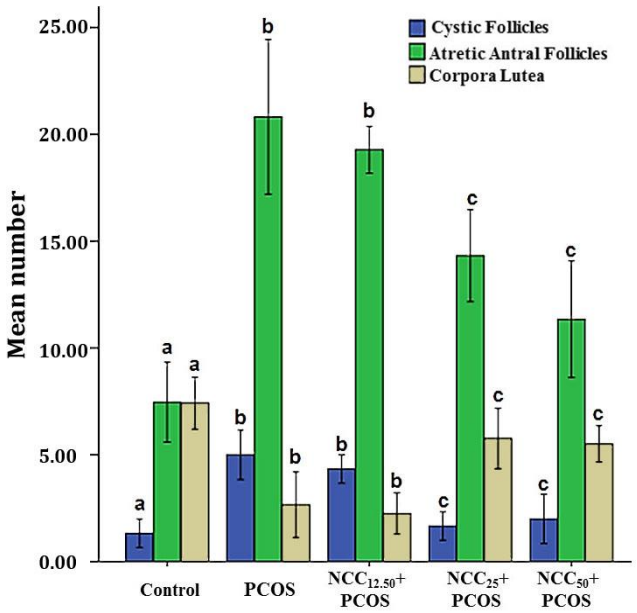

Fig. 2. Histological findings in all experimental groups. PCOS: Polycystic ovary syndrome; NCC: Nanocurcumin.
[abc] Values with different superscripts within one column differ significantly at $p < 0.05$.

Embryological findings. Detailed information regarding IVF and *in vitro* pre-implantation embryo development is furnished in Table 2. Mice in PCOS group showed considerable reductions in zygote, two-cell embryo, morula and blastocyst percentages compared to those in control group. Post-PCOS treatment with NCC at the doses of 25.00 and 50.00 mg kg^{-1} led to improved IVF rate and promoted *in vitro* early embryonic development being manifested as an obvious upsurge in fertilization as well as two-cell embryo and blastocyst formation rates.

Table 1. Biochemical profile in all experimental groups.

Group	MDA (µmol per g tissue)	TAC (µmol per g tissue)	CAT (µmol per g tissue)	Testosterone (ng mL^{-1})
Control	1.06 ± 0.13[a]	2.97 ± 0.12[a]	26.14 ± 2.73[a]	0.64 ± 0.06[a]
PCOS	2.59 ± 0.24[b]	1.05 ± 0.17[b]	9.28 ± 0.94[b]	1.95 ± 0.04[b]
$NCC_{12.50}$ + PCOS	2.62 ± 0.37[b]	1.83 ± 0.01[b]	13.04 ± 1.83[b]	1.65 ± 0.12[b]
NCC_{25} + PCOS	2.16 ± 0.22[b]	2.53 ± 0.18[c]	21.04 ± 2.19[c]	1.01 ± 0.28[c]
NCC_{50} + PCOS	1.94 ± 0.22[b]	2.41 ± 0.08[c]	16.73 ± 1.58[c]	0.93 ± 0.11[c]

PCOS: Polycystic ovary syndrome; NCC: Nanocurcumin; MDA: Malondialdehyde; TAC: Total anti-oxidant capacity; CAT: Catalase.
[abc] Values with different superscripts within one column differ significantly at $p < 0.05$.

Table 2. Embryological findings in all experimental groups.

Group	Zygote (%)	Two-cell embryo (%)	Morula (%)	Blastocyst (%)
Control	93.16 ± 7.64[a]	90.04 ± 11.27[a]	0.64 ± 0.06[a]	84.04 ± 5.37[a]
PCOS	56.09 ± 4.18[b]	64.09 ± 9.11[b]	1.95 ± 0.04[b]	47.02 ± 4.94[b]
$NCC_{12.50}$ + PCOS	61.33 ± 6.12[b]	69.22 ± 9.08[b]	1.65 ± 0.12[b]	52.38 ± 7.27[b]
NCC_{25} + PCOS	75.29 ± 4.39[c]	74.09 ± 6.30[c]	1.01 ± 0.28[b]	69.84 ± 5.10[c]
NCC_{50} + PCOS	79.26 ± 8.12[c]	71.37 ± 6.75[c]	0.93 ± 0.11[b]	72.22 ± 6.15[c]

PCOS: Polycystic ovary syndrome; NCC: Nanocurcumin.
[abc] Values with different superscripts within one column differ significantly at $p < 0.05$.

Discussion

This study evinced that PCOS caused ovarian histo-architectural disorganization and oxidant/anti-oxidant imbalance, hyperandrogenism and IVF success and pre-implantation embryo development retardation in mice. In concurrence with our findings, former reports have emphasized that PCOS results in reactive oxygen species over-generation and hormonal dysregulation leading to ovarian histomorphological disarrangement and reproductive disorders.[12,20] It has also been widely recorded that PCOS-related hyperandrogenemia induces oxidative stress-evoked fertilization rate reduction and embryo developmental arrest.[20,21] Further, it is well-documented that hyperandrogenemia-linked dyslipidemia plays a fundamental role in pathophysiology of PCOS and the connection between dyslipidemia and oxidative stress has been demonstrated previously.[22,23] Correspondingly, oxidative stress triggers pro-inflammatory cytokines production and inflammatory responses have been associated with hyperandrogenism.[24,25]

Our observations revealed that NCC administration at the doses of 25.00 and 50.00 mg kg^{-1} reinstated PCOS-associated ovarian, endocrine and embryological disturbances in mice, supporting earlier reports suggesting NCC as a promising therapeutic candidate due to its enhanced pharmacokinetic properties owing to the nanoencapsulation.[10] Similarly, recent evidence has also indicated that NCC is more effective in tissue damage, oxidative stress, inflammation and apoptosis mitigation than CC.[26] Correspondingly, it has been shown lately that NCC palliates oxidative and endoplasmic reticulum stresses induced by long-term carbohydrate intake diet, being attributed to its nanoencapsulation-related augmented free radicals scavenging abilities.[27] In congruity with the earlier reports, our investigations highlighted that NCC extenuated reproductive complications in PCOS condition through androgen profile restoration and anti-oxidant defense mechanisms reinforcement.[7,28] In this study, the repro-protective effects of NCC in murine model of PCOS may be ascribed to its multiple biological functions including anti-oxidant, anti-inflammatory, anti-fibrosis, anti-hyperlipidemic, anti-apoptotic and estrogenic activities.[9,10,13,28] Likewise, in support of our findings, very recent report has stated that NCC preserves renal function and hematological profile in 7,12-dimethylbenz[a] anthracene-induced ovarian cancer being treated with cisplatin through its anti-oxidant and anti-inflammatory activities in rats.[29]

In toto, the present *in vivo* and *in vitro* findings add continued weight to the evidence that NCC has potential repro-protective properties, particularly against ovo-toxicities. However, since CC nanoparticles do not function in a tissue-specific manner, larger attempts are needed to develop tissue-specific nanomedicine delivery strategies to increase their biosafety and bio-efficacy. Further molecular mechanisms-oriented studies are also needed to unveil the precise functional nature of NCC.

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Conflict of interest

The authors declare that there are no known competing interests/personal relationships that could have appeared to influence the work reported in this article.

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