

IN-VITRO PHARMACOLOGICAL EVALUATION OF MORINGA OLEIFERA LEAF EXTRACT AND GUAR GUM COMBINATION IN A CELLULAR MODEL OF POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is characterised by hyperandrogenism, abnormal follicular development, insulin resistance, oxidative stress, and chronic low-grade inflammation [1–6]. Moringa oleifera leaves contain antioxidant, anti-inflammatory, and metabolically active phytoconstituents [9–15], while guar gum is a soluble galactomannan fibre with beneficial effects on glucose and lipid metabolism [16–20]. Their combined effect on PCOS-associated cellular dysfunction remains insufficiently investigated. Objective: To evaluate, in vitro, the individual and combined effects of Moringa oleifera leaf extract and guar gum on PCOS-associated cellular dysfunction using the KGN human granulosa-like cell model [7,21,22]. Methods: A validated, testosterone-induced PCOS-relevant KGN cell phenotype [21] was treated with Moringa oleifera leaf extract, guar gum, or their combination, alongside a positive control, at concentrations derived from cytotoxicity and dose–response studies. Cell viability, oxidative stress markers, inflammatory mediators, and steroidogenic markers (testosterone, estradiol, progesterone) were assessed using validated biochemical and molecular techniques. Results: Illustrative/placeholder values are shown in this draft (Section 7, Tables 1–4) pending completion of the actual assays; these figures must be replaced with genuine experimental data before submission. Conclusion: This study outlines an animal-free, mechanistic assessment of the pharmacological potential of Moringa oleifera and guar gum, individually and in combination, against PCOS-associated granulosa-cell dysfunction.

Keywords: Polycystic ovary syndrome; Moringa oleifera; guar gum; KGN cells; granulosa cells; oxidative stress; steroidogenesis; insulin resistance; in-vitro pharmacology.

1. Introduction

Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders in women of reproductive age and a leading cause of anovulatory infertility [1,8]. Hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology are the principal diagnostic features, frequently accompanied by insulin resistance, dyslipidaemia, oxidative stress, and chronic low-grade inflammation [1,2]. Insulin resistance and compensatory hyperinsulinaemia enhance ovarian androgen production, and excess androgen impairs follicular development [1,8]; granulosa cells, central to follicular development and steroidogenesis, show abnormal function and altered steroidogenic activity in PCOS [7,21,22]. Increased ROS generation and inadequate antioxidant defence further impair granulosa-cell viability, mitochondrial function, and steroidogenesis, interacting with inflammation and insulin resistance [2–6]. Current management (lifestyle intervention, insulin sensitisers, hormonal contraceptives, anti-androgens, ovulation induction) is tailored to phenotype, motivating continued interest in complementary multi-target agents.

Moringa oleifera Lam. leaves contain flavonoids, phenolics, glucosinolates, vitamins, and minerals with antioxidant, anti-inflammatory, and metabolic activity [13–15], improving insulin-related abnormalities and folliculogenesis in a testosterone propionate-induced PCOS rat model [9] and oxidative stress/inflammation in letrozole-induced PCOS rats [10]. Guar gum, a soluble galactomannan, influences gastrointestinal glucose absorption and lipid metabolism [16–20]; its direct cellular effect on ovarian cells (versus its established gut-mediated mechanism) is exploratory here. The two agents may act through complementary mechanisms — *Moringa* via antioxidant/anti-inflammatory/steroidogenic modulation, guar gum via metabolic effects — and their combined cellular effect in a validated ovarian model remains inadequately investigated.

Hypothesis: *Moringa oleifera* leaf extract and guar gum, individually and particularly in combination, attenuate PCOS-associated cellular dysfunction by modulating oxidative stress, inflammatory signalling, and steroidogenic activity in KGN cells.

2. Aim

To evaluate the in-vitro pharmacological effects of *Moringa oleifera* leaf extract and guar gum, individually and in combination, on PCOS-associated cellular dysfunction in KGN human granulosa-like cells.

3. Objectives

- To prepare/standardise *Moringa oleifera* leaf extract and evaluate cytocompatibility of the extract and guar gum in KGN cells.
- To establish and validate a reproducible, testosterone-induced PCOS-relevant cellular model.
- To investigate the individual and combined effects of *Moringa* extract and guar gum on oxidative stress, steroidogenic markers (testosterone, estradiol, progesterone), and inflammatory markers (TNF- α , IL-6, IL-1 β , NF- κ B).
- To investigate insulin/metabolic signalling where technically feasible.
- To determine, through formal combination analysis, whether the combination produces an enhanced or synergistic effect relative to individual treatments.

4. Materials and Methods

4.1 Cell Line, Extract, and Guar Gum

KGN human granulosa-like tumour cells, established by Nishi et al. [7] and widely used for PCOS-relevant granulosa-cell studies [21,22], are the primary in-vitro model (source, catalogue/passage number, and authentication to be recorded), maintained in complete DMEM/F-12 with fetal bovine serum and antibiotics at 37 °C, 5% CO₂. Authenticated *Moringa oleifera* leaves are washed, shade-dried, powdered, and extracted by a validated procedure, standardised by extraction yield, phytochemical screening, total phenolic/flavonoid content, and HPLC/LC–MS fingerprinting where available [13–15]. Pharmaceutical/food-grade guar gum is used, with manufacturer, batch, grade, and purity recorded [16–20].

4.2 Cytotoxicity and PCOS Model Validation

A concentration range of *Moringa* extract and guar gum is screened in KGN cells by a validated viability assay (MTT/resazurin/CCK-8); prior rodent doses [9] are not extrapolated directly to culture, and concentrations are expressed in μ g/mL or μ M from dose–response curves. A PCOS-like phenotype is induced using a validated androgenic stimulus (testosterone, per established protocols [21]) or an oxidative/inflammatory stimulus, selected by pilot experiments for reproducibility without excessive cytotoxicity, and validated by steroidogenic change, ROS generation, inflammatory markers, and steroidogenic enzyme expression before treatment evaluation.

4.3 Experimental Groups

Group	Treatment
I	Normal control cells
II	PCOS-model control
III	PCOS model + Moringa (low concentration)
IV	PCOS model + Moringa (high concentration)
V	PCOS model + guar gum
VI	PCOS model + Moringa + guar gum (combination)
VII	PCOS model + standard/positive control

Concentrations for all groups, including the combination, will be selected from individual concentration–response curves at non-cytotoxic, pharmacologically relevant levels, followed by a formal combination-index analysis [27] rather than by direct translation of prior animal doses.

4.4 Endpoints

Cell viability is expressed as percentage viability relative to untreated controls. Oxidative stress is accessed via intracellular ROS (fluorescent assay), GSH, SOD, catalase, and MDA where available [2–6]. Steroidogenic assessment includes testosterone, estradiol, and progesterone in culture supernatants by validated immunoassay, plus CYP17A1, CYP19A1, 3 β -HSD, and StAR expression where facilities permit. Inflammatory markers (TNF- α , IL-6, IL-1 β , NF- κ B signalling) and insulin/metabolic signalling (GLUT4, IRS-1, PI3K, AKT, AMPK) are assessed per laboratory capability. Molecular investigation by qPCR/western blot targets Nrf2/HO-1, NF- κ B, CYP17A1/CYP19A1/StAR, and IRS-1/PI3K/AKT/GLUT4.

5. Proposed Mechanism

The working hypothesis is that Moringa-derived bioactive constituents contribute antioxidant, anti-inflammatory, and steroidogenic modulation, while guar gum contributes complementary metabolic/cellular effects; together these may reduce oxidative stress and inflammatory signalling, modulate steroidogenesis, and improve insulin-related signalling, thereby improving granulosa-cell function. This mechanism remains proposed and will only be supported if the corresponding molecular endpoints are demonstrated experimentally.

6. Statistical Analysis

All experiments will include independent biological replicates, with results expressed as mean $\pm$ SD or SEM as prespecified. One-way ANOVA with an appropriate post-hoc test will be used for multi-

group comparisons after checking assumptions; nonlinear regression will be applied to concentration–response data. A significance threshold of $p < 0.05$ will be used. For the combination group, a formal combination-index approach (e.g., the Chou–Talalay method [27]) will be applied before any claim of synergism; in the absence of such analysis, the combination will be described only as producing 'an enhanced effect' relative to individual treatments, not as 'synergistic'.

7. Anticipated Results

The final result present cell-viability, oxidative-stress, steroidogenic, and molecular-marker data in tabulated form (viability by group and concentration; ROS/MDA/SOD/GSH by group; testosterone/estradiol/progesterone by group; and relative expression of CYP17A1, CYP19A1, Nrf2, NF- κ B, and AKT by group), together with the corresponding statistical analysis.

Table 1. Effect of treatments on KGN cell viability

Group	Cell viability (%)
Normal control	100.2 $\pm$ 3.2
PCOS model	75.7 $\pm$ 4.1
Moringa	89.4 $\pm$ 3.6
Guar gum	85.9 $\pm$ 3.0
Combination	99.4 $\pm$ 2.8
Standard	91.6 $\pm$ 3.1

Table 2. Effect of treatments on oxidative stress parameters in KGN cells

Group	ROS (RFU)	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH (μ mol/mg protein)
Control	100.2 $\pm$ 4.1	1.84 $\pm$ 0.12	9.34 $\pm$ 0.42	6.72 $\pm$ 0.35
PCOS model	168.7 $\pm$ 6.3	3.46 $\pm$ 0.18	4.92 $\pm$ 0.31	3.83 $\pm$ 0.27
Moringa	128.4 $\pm$ 5.2	2.51 $\pm$ 0.15	6.80 $\pm$ 0.36	5.47 $\pm$ 0.31
Guar gum	140.8 $\pm$ 5.7	2.76 $\pm$ 0.16	6.33 $\pm$ 0.34	5.04 $\pm$ 0.29
Combination	111.6 $\pm$ 4.6	2.03 $\pm$ 0.13	7.97 $\pm$ 0.39	6.27 $\pm$ 0.33
Standard	116.4 $\pm$ 4.8	2.14 $\pm$ 0.14	7.67 $\pm$ 0.37	6.07 $\pm$ 0.32

Values are shown as mean $\pm$ SD (illustrative; $n = 6$).

Table 3. Effect of *Moringa oleifera* and guar gum on steroidogenesis in KGN cells

Group	Testosterone (ng/mL)	Estradiol (ng/mL)	Progesterone (ng/mL)
Control	0.82 ± 0.06	1.69 ± 0.11	2.95 ± 0.18
PCOS model	1.76 ± 0.10	0.95 ± 0.08	1.62 ± 0.14
Moringa	1.23 ± 0.08	1.35 ± 0.10	2.33 ± 0.16
Guar gum	1.38 ± 0.09	1.26 ± 0.09	2.12 ± 0.15
Combination	0.94 ± 0.07	1.58 ± 0.10	2.75 ± 0.17
Standard	1.03 ± 0.07	1.48 ± 0.10	2.62 ± 0.16

Values are expressed as mean ± SD; illustrative values only (n = 6).

Table 4. Effect of *Moringa oleifera* and guar gum on molecular markers associated with PCOS

Molecular marker	Control	PCOS model	Moringa	Guar gum	Combination
CYP17A1	1.00 ± 0.05	2.38 ± 0.14	1.57 ± 0.10	1.76 ± 0.11	1.17 ± 0.07
CYP19A1	1.00 ± 0.06	0.49 ± 0.04	0.76 ± 0.05	0.68 ± 0.05	0.98 ± 0.06
Nrf2	1.00 ± 0.05	0.63 ± 0.04	1.27 ± 0.08	1.16 ± 0.07	1.48 ± 0.09
NF-κB	1.00 ± 0.05	2.53 ± 0.15	1.64 ± 0.10	1.83 ± 0.12	1.21 ± 0.08
AKT	1.00 ± 0.05	0.58 ± 0.04	0.79 ± 0.05	0.73 ± 0.05	0.95 ± 0.06

Values are expressed as relative fold change (mean ± SD; illustrative values; n = 6). Control = 1.00.

8. Discussion

KGN granulosa-like cells provide an experimentally accessible, animal-free model for PCOS-relevant granulosa-cell mechanisms [7,21,22]. Successful establishment of the PCOS-like phenotype is indicated by increased oxidative stress and altered steroidogenesis relative to controls [2–6]. A reduction in ROS following *Moringa* treatment is consistent with its reported antioxidant activity in in-vivo PCOS models [9,10] and other oxidative-stress contexts [12,13]; testosterone/estradiol changes are interpreted alongside CYP17A1/CYP19A1 expression. Guar gum's effects require cautious interpretation in an isolated ovarian-cell system, since its principal action relates to gut-mediated soluble-fibre effects [16–20]; any direct KGN-cell effect is a cellular pharmacological observation, not a reproduction of its established mechanism. Greater improvement with the combination than with either agent alone would suggest complementary activity, though formal synergy analysis [27] is required before describing the interaction as genuinely synergistic rather than additive.

9. Conclusion

This study outlines an animal-free, in-vitro pharmacological approach for evaluating *Moringa oleifera* leaf extract and guar gum, individually and in combination, against PCOS-associated granulosa-cell dysfunction, with emphasis on oxidative stress, steroidogenesis, inflammation, and metabolic signalling. A combination-analysis-supported enhancement over either individual treatment would provide a mechanistic basis for subsequent preclinical investigation.

10. Limitations

An in-vitro granulosa-cell system cannot reproduce the full endocrine and metabolic complexity of PCOS, and cannot directly assess oestrous cyclicity, whole-body insulin resistance, hypothalamic–pituitary LH/FSH secretion, intact-organism ovarian morphology, gastrointestinal effects of guar gum, or systemic pharmacokinetics. Findings should be interpreted as cellular mechanistic evidence rather than proof of clinical efficacy.

11. Future Scope

Formal dose–response and synergy analysis of the combination [27]; Nrf2/HO-1, PI3K/AKT/GLUT4, AMPK, and NF- κ B pathway studies; CYP17A1/CYP19A1 expression profiling; mitochondrial function and apoptosis assays; transcriptomic/metabolomic profiling; and 3-D ovarian/organoid models with subsequent validated preclinical (in-vivo) studies.

Declarations

Funding: Rs. 90,000/-.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: As this is a cell-line (non-animal, non-human-subject) protocol, institutional biosafety clearance is required prior to experimentation.

References

1. Rojas J, Chávez M, Olivar L, Rojas M, Morillo J, Mejías J, Calvo M, Bermúdez V. Polycystic ovary syndrome, insulin resistance, and obesity: navigating the pathophysiologic labyrinth. *Int J Reprod Med.* 2014;2014:719050.
2. Reactive oxygen species in polycystic ovary syndrome: mechanistic insights into pathogenesis and therapeutic opportunities. *Reprod Toxicol.* 2025. Available from: PMC12304726.
3. Liu S, Jia Y, Meng S, Luo Y, Yang Q, Pan Z. Mechanisms of and potential medications for oxidative stress in ovarian granulosa cells: a review. *Int J Mol Sci.* 2023;24(11):9205. doi:10.3390/ijms24119205.

4. Gao Y, Zou Y, Wu G, Zheng L. Oxidative stress and mitochondrial dysfunction of granulosa cells in polycystic ovarian syndrome. *Front Med.* 2023;10:1193749. doi:10.3389/fmed.2023.1193749.
5. Zhang M, et al. Oxidative stress and energy metabolism abnormalities in polycystic ovary syndrome: from mechanisms to therapeutic strategies. *Reprod Biol Endocrinol.* 2024;22:150.
6. Polycystic ovary syndrome and oxidative stress-from bench to bedside. *Int J Mol Sci.* 2023;24(18):14126. doi:10.3390/ijms241814126.
7. Nishi Y, Yanase T, Mu Y, Oba K, Nomura M, Okabe T, Goto K, Takayanagi R, Kashimura Y, Haji M, Nawata H. Establishment and characterization of a steroidogenic human granulosa-like tumor cell line, KGN, that expresses functional follicle-stimulating hormone receptor. *Endocrinology.* 2001;142(1):437-445. doi:10.1210/endo.142.1.7862.
8. Deswal R, Yadav A, Dang AS. Sex hormone binding globulin - an important biomarker for predicting PCOS risk: a systematic review and meta-analysis. *Syst Biol Reprod Med.* 2018;64(1):12-24.
9. Amelia D, Santoso B, Purwanto B, Miftahussurur M, Joewono HT, Budiono. Effects of *Moringa oleifera* on insulin levels and folliculogenesis in polycystic ovary syndrome model with insulin resistance. *Immunol Endocr Metab Agents Med Chem.* 2018;18(1):22-30. doi:10.2174/1871522218666180426100754.
10. Wu G, Li Y, Sun J, Wang F, Xu M, Fang X, et al. *Moringa oleifera* leaf supplementation relieves oxidative stress and regulates intestinal flora to ameliorate polycystic ovary syndrome in letrozole-induced rats. *Food Sci Nutr.* 2023;11(11):6893-6905. doi:10.1002/fsn3.3473.
11. Amelia D, et al. The effect of *Moringa oleifera* leaf extract on theca cell in polycystic ovary syndrome model with insulin resistance. *Proc SIHC.* 2020.
12. Albrahim T, Binobead MA. *Moringa oleifera* leaf extract improves the impact of high dietary intake of monosodium glutamate-induced liver toxicity, oxidative stress, genotoxicity, DNA damage, and PCNA alterations in male rats. *Oxid Med Cell Longev.* 2018;2018:4501097.
13. Kushwaha S, Chawla P, Kochhar A. Effect of supplementation of drumstick (*Moringa oleifera*) and amaranth (*Amaranthus tricolor*) leaves powder on antioxidant profile and oxidative status among postmenopausal women. *J Food Sci Technol.* 2014;51(11):3464-3469.
14. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. *Moringa oleifera* seeds and oil: characteristics and uses for human health. *Int J Mol Sci.* 2016;17(12):2141.
15. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. *Antioxidants.* 2017;6(4):91.

16. Butt MS, Shahzadi N, Sharif MK, Nasir M. Guar gum: a miracle therapy for hypercholesterolemia, hyperglycemia and obesity. *Crit Rev Food Sci Nutr.* 2007;47(4):389-396.
17. Grover JK, Kumar V, Yadav SP, Vats V. Guar gum in insulin-dependent diabetes: effects on glycemic control and serum lipoproteins. *Am J Clin Nutr.* 1993.
18. Aro A, Uusitupa M, Voutilainen E, Hersio K, Korhonen T, Siitonen O. Improved diabetic control and hypocholesterolaemic effect induced by long-term dietary supplementation with guar gum in type 2 (insulin-independent) diabetes. *Diabetologia.* 1981;21(1):29-33.
19. Uusitupa M, Siitonen O, Savolainen K, Silvasti M, Penttilä I, Parviainen M. Metabolic and nutritional effects of long-term use of guar gum in the treatment of noninsulin-dependent diabetes of poor metabolic control. *Am J Clin Nutr.* 1989;49(2):345-351.
20. Ahmadi K, et al. The effects of guar gum supplementation on glycemic control, body mass and blood pressure in adults: a GRADE-assessed systematic review and meta-analysis of randomized clinical trials. *Nutr Res.* 2023.
21. Liu S, et al. Circ_FURIN knockdown assuages testosterone-induced human ovarian granulosa-like tumor cell disorders by sponging miR-423-5p to reduce MTM1 expression in polycystic ovary syndrome. *J Ovarian Res.* 2022;15:16.
22. Havelock JC, Rainey WE, Carr BR. Ovarian granulosa cell lines. *Mol Cell Endocrinol.* 2004;228(1-2):67-78.
23. Tripathi KD. *Essentials of Medical Pharmacology.* 8th ed. New Delhi: Jaypee Brothers Medical Publishers; 2019.
24. Brunton LL, Hilal-Dandan R, Knollmann BC, editors. *Goodman & Gilman's The Pharmacological Basis of Therapeutics.* 13th ed. New York: McGraw-Hill; 2017.
25. Katzung BG, Vanderah TW, editors. *Basic and Clinical Pharmacology.* 15th ed. New York: McGraw-Hill; 2021.
26. Chou TC. *Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug com*