

Peer Review

Review of: "Evaluation of Antidiabetic Potential of Gymnema Sylvestre and Metformin Combination in Streptozotocin-Induced Diabetic Rats"

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Comment 1: The age range "6-8 weeks" is insufficiently precise for metabolic studies. Rats exhibit significant physiological differences between 42 and 56 days of age. Exact mean age $\pm$ SD should be reported to ensure reproducibility.

Comment 2: High-Fat Diet Composition, the HFD composition (60% fat, 20% protein, 20% carbohydrates) lacks critical details: Source of fats (e.g., lard vs. vegetable oil), Fatty acid profile (SFA/MUFA/PUFA ratios), Cholesterol content

These omissions prevent accurate model replication and interpretation of lipid-related outcomes.

Comment 3: The STZ dose (25 mg/kg $\times$ 2 injections) is suboptimal for Sprague Dawley rats. Published protocols require 35-45 mg/kg for consistent hyperglycemia (>300 mg/dL). The current regimen risks incomplete β -cell destruction.

Comment 4: The xylazine dose (100 mg/kg) is 10-fold higher than the maximum safe dose (5-10 mg/kg). This error likely caused mortality or severe physiological stress, confounding results.

Comment 5: Centrifugation at 7000g for plasma separation causes hemolysis. Standard protocols require 1500-2000g.

Comment 6: Two-way ANOVA is inappropriate for repeated measures. Mixed-effects modeling or RM-ANOVA should be used.

Comment 7: Reported creatinine reduction (Met group: 1.85 $\rightarrow$ 0.44 mg/dL) exceeds physiological limits. Normal rat creatinine is 0.2-0.8 mg/dL. Values <0.5 mg/dL suggest analytical error or unit confusion

($\mu\text{mol/L}$ vs mg/dL).

Comment 8: No insulin measurements were performed despite using an insulin resistance model. Without HOMA-IR calculations, the T2DM phenotype remains unverified.

Comment 9: *Gymnema sylvestre* extract lacks phytochemical characterization. Absence of gymnemic acid quantification prevents dose validation and reproducibility.

Comment 10: No histopathology of pancreas/liver/kidneys. Without β -cell mass assessment, STZ efficacy cannot be confirmed.

Comment 11: Claims of GS's "inhibition of glucose absorption" lack supporting data (e.g., OGTT, intestinal GLUT expression). Metformin's "kidney protection" is unsupported without renal histopathology.

Declarations

Potential competing interests: No potential competing interests to declare.