

REVIEWER'S REPORT

Manuscript No.: IJAR-59274

Title: ,In Vivo Evaluation of the Antihyperglycemic Activity of Polyherbal Unani Formulation (Tablet Dolabi) Using an Oral Glucose Tolerance Test in Swiss Albino Mice

Recommendation:

Accept as it is

Accept after minor revision.....

Accept after major revision ...YES.....

Do not accept (*Reasons below*)

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality			✓	
Clarity		✓		
Significance		✓		

Reviewer's ID: JPR-094

Detailed Reviewer's Report

Reviewer Report

1. Overall assessment

The manuscript addresses the **acute antihyperglycemic/glucose-tolerance effects of Tablet Dolabi**, a polyherbal Unani formulation, using an OGTT in Swiss albino mice. The topic is relevant to experimental pharmacology and traditional-medicine research. The manuscript has a clear basic experimental concept, includes a glucose-loaded control and a conventional reference drug, and attempts to compare two doses of the formulation.

However, in its present form, the manuscript has **substantial methodological, statistical, reporting, and interpretational deficiencies**. Most importantly, several details necessary to establish the validity and reproducibility of the animal experiment are missing. The conclusions regarding "synergistic" effects and comparison with glibenclamide are stronger than what can be supported by the experimental design. The study uses **healthy mice rather than a diabetic model**, making the term "antidiabetic" or claims of therapeutic efficacy inappropriate at this stage.

Recommendation: Major Revision

2. Strengths of the manuscript

2.1 Relevant research topic

The evaluation of a traditional polyherbal Unani formulation using an experimental pharmacological model is scientifically relevant and potentially valuable for generating preliminary evidence.

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2.2 Inclusion of a standard drug

The inclusion of **glibenclamide** as a positive/reference control is an important strength because it provides a pharmacological comparator.

2.3 Dose comparison

The use of two doses of Tablet Dolabi (200 and 400 mg/kg) allows preliminary assessment of a possible dose-response relationship.

2.4 Multiple OGTT time points

Blood glucose was measured at 0, 30, 60, 90 and 120 min. This provides a temporal profile rather than relying on a single glucose measurement.

2.5 Description of formulation composition

The manuscript provides the amount of each active ingredient per tablet:

- Gymnema sylvestre – 55 mg
- Bambusa bambos – 118 mg
- Rumex vesicarius – 79 mg
- Asphaltum – 35 mg
- Acacia arabica – 171 mg

This is useful information for reproducibility, although further authentication and standardization information is needed.

2.6 Ethical considerations are mentioned

The manuscript states that IAEC approval was obtained and that animal welfare measures were followed. This is essential for an in-vivo study.

2.7 Limitations are partially acknowledged

The authors appropriately acknowledge that the experiment was conducted in healthy animals and recommend future studies in diabetic models.

3. Major weaknesses / concerns

A. Experimental design

3.1 Very small sample size

Each group contains only **n = 5 animals**. Such a small sample can substantially limit statistical power and the reliability of between-group comparisons.

The authors should provide:

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- sample-size calculation/power justification;
- expected effect size;
- significance level;
- statistical power;
- whether $n=5$ was determined before the experiment.

Without this information, the claim of significant treatment effects should be interpreted cautiously.

3.2 Healthy mice are not an appropriate model for establishing antidiabetic efficacy

This is perhaps the most important scientific limitation.

The animals are described as healthy Swiss albino mice. An OGTT in healthy animals can demonstrate an effect on **glucose tolerance**, but it does not establish efficacy against diabetes mellitus.

Therefore, terminology such as:

"antidiabetic activity"

or

"therapeutic utility for diabetes"

should be substantially moderated.

The study can reasonably be presented as an **acute glucose-tolerance/antihyperglycemic screening study in healthy mice**.

A diabetic model such as streptozotocin- or diet-induced diabetes would be necessary for stronger claims concerning antidiabetic activity.

3.3 Sex of animals is not adequately controlled or reported

The manuscript states:

"both male and female"

but does not report the number of males and females in each group.

Sex can influence glucose metabolism and pharmacological responses. The authors should report:

- number of male/female animals;
- allocation of sex across groups;
- whether sex was included as a biological variable.

If the experiment was not designed to examine sex differences, the authors should at least explain how balanced allocation was achieved.

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3.4 Randomization is inadequately described

The manuscript states that animals were randomly divided but does not explain the randomization method.

The authors should state how randomization was performed and whether allocation was concealed.

3.5 Blinding is not reported

There is no information regarding blinding of investigators who administered treatments or measured blood glucose.

The authors should clarify whether:

- treatment allocation was blinded;
- glucose measurements were performed by a blinded investigator;
- data analysis was blinded.

4. Important methodological problems

4.1 Treatment timing is unclear

The manuscript states:

"After the predetermined treatment period, glucose solution was administered..."

but the actual interval between Tablet Dolabi/glibenclamide administration and glucose administration is not clearly specified.

This is critical in an OGTT.

The manuscript should clearly state, for example:

Treatment → waiting period → glucose administration → glucose measurements

The exact time interval must be provided.

4.2 Glucose dose of 4 g/kg requires justification

The study uses **4 g/kg glucose**, which is a relatively high glucose challenge. The authors should provide a clear scientific justification and cite the exact protocol followed.

They should also explain whether the dose is appropriate for the particular mouse strain, age and experimental conditions.

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4.3 Vehicle is inadequately specified

The manuscript says:

"distilled water or 0.5% carboxymethyl cellulose (CMC)"

This is problematic because the actual vehicle must be clearly specified.

The authors should identify:

- exact vehicle used;
- volume administered;
- whether the same vehicle was administered to all groups;
- concentration of CMC, if applicable.

"Distilled water or CMC" is not acceptable in a reproducible experimental protocol.

4.4 Formulation preparation requires clarification

The authors state that the commercial tablet was powdered and suspended. However, important details are absent:

- manufacturer;
- batch number;
- manufacturing/expiry date;
- source of Tablet Dolabi;
- authentication of ingredients;
- quality-control specifications;
- extraction/preparation process, if any;
- pH or physical characteristics of the suspension;
- homogeneity/stability of the suspension.

For a commercial polyherbal formulation, these details are particularly important.

4.5 Herbal ingredients are not chemically standardized

The manuscript attributes activity to gymnemic acids, flavonoids, phenolics, tannins, etc., but there is no evidence that these constituents were actually quantified in the Tablet Dolabi used in the experiment.

Therefore, statements suggesting that particular constituents caused the observed activity remain **hypothetical**.

The authors should clearly distinguish between:

reported constituents of individual plants

and

experimentally demonstrated constituents of the tested Tablet Dolabi formulation.

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5. Acute toxicity section is insufficient

Section 2.6 states that an acute toxicity study was performed according to OECD 423, but the actual results are not presented.

The authors should provide:

- number of animals used;
- dose levels tested;
- observation period;
- signs/symptoms observed;
- mortality;
- body-weight changes, if measured;
- final conclusion regarding toxicity;
- basis for selecting 200 and 400 mg/kg.

Simply stating that toxicity assessment was conducted is insufficient.

Also, if OECD 423 was actually followed, the experimental procedure should be reported sufficiently to demonstrate compliance.

6. Statistical concerns

6.1 Statistical method may not be appropriate for repeated measurements

Blood glucose is measured repeatedly in the **same animals** at 0, 30, 60, 90 and 120 min.

The manuscript states that one-way ANOVA followed by Tukey's test was used.

This does not adequately account for the repeated measurements within the same animal.

A more appropriate analysis would generally involve a **repeated-measures approach**, mixed-effects model, or another statistically justified method accounting for within-animal correlation.

The authors should consult a statistician and reanalyse the data appropriately.

6.2 Exact p-values are absent

The manuscript reports:

$p < 0.001$

but does not provide exact p-values.

The authors should provide exact p-values wherever possible, together with the statistical test used.

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6.3 Statistical comparison is unclear

The table says that superscript "a" represents:

$p < 0.001$ compared to the respective control group.

However, it is unclear whether this means:

- comparison with glucose control at each time point;
- comparison at 120 min only;
- comparison with normal control;
- comparison of treatment groups with Group II.

This needs to be explicitly stated.

6.4 Multiple time-point comparisons need correction

If multiple groups and multiple time points are compared, the statistical analysis needs to appropriately address multiple comparisons.

6.5 Mean $\pm$ SEM with n=5 may not adequately communicate variability

The authors should consider presenting individual animal data or at least mean $\pm$ SD in addition to SEM.

For an animal study with n=5, individual data points would substantially improve transparency.

7. Major concern regarding the "percentage reduction" calculation

The manuscript reports:

- Glibenclamide: 32%
- Tablet Dolabi 200 mg/kg: 23%
- Tablet Dolabi 400 mg/kg: 30%

However, the equation given is:

$$\text{Percentage Reduction} = [(G_c - G_t)/G_c] \times 100$$

where G_c and G_t appear to be blood glucose concentrations.

This calculation is potentially problematic because the manuscript describes "reduction in blood glucose elevation," but the equation appears to use absolute glucose concentrations.

The authors should clearly define whether percentage reduction was calculated using:

- absolute glucose concentration;
- change from baseline;

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- glucose excursion above baseline;
- peak glucose;
- 120-min glucose;
- AUC.

For an OGTT, **glucose AUC (or incremental AUC)** is generally much more informative than calculating a single percentage from one time point.

The authors should therefore consider calculating and statistically comparing:

AUC_{0–120 min}

and preferably:

incremental AUC (iAUC).

8. The reported 23%, 30% and 32% values need verification

The numerical basis of these percentages is not adequately explained.

For example, the high-dose group has:

- glucose control at 120 min = 145 mg/dL
- treatment group at 120 min = 102 mg/dL.

Using the stated formula:

$$[(145-102)/145] \times 100 = 29.7\%$$

gives approximately **29.7%**, which corresponds to the reported 30%.

However, this represents reduction relative to the glucose-control concentration at 120 min—not necessarily "reduction in glucose elevation."

The authors should revise the terminology and calculation methodology consistently throughout the manuscript.

9. Claims of "synergy" are not demonstrated

The manuscript repeatedly uses expressions such as:

"synergistic actions"

and

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"synergistic effects of bioactive constituents."

However, no experiment was conducted to establish synergy.

To demonstrate synergy, one would generally need experimental designs involving individual components and combinations, with an appropriate quantitative interaction analysis.

The present study only evaluates the **complete formulation**.

Therefore, the manuscript should use more cautious language such as:

- "possible complementary effects"
- "potential contribution of multiple constituents"
- "hypothesized multi-component activity"

rather than claiming demonstrated synergy.

10. Comparison with glibenclamide should be interpreted cautiously

The manuscript states that the 400 mg/kg dose:

"comparing favorably to" glibenclamide

because the reported reductions are 30% and 32%.

This interpretation is too strong.

Numerical similarity does not demonstrate equivalence or comparable pharmacological efficacy.

The authors should avoid implying that Tablet Dolabi is equivalent to glibenclamide unless a statistically appropriate comparison demonstrates this.

Moreover, glibenclamide acts through a defined insulin-secretagogue mechanism, while the present study did not measure insulin.

11. Mechanistic conclusions are speculative

The discussion attributes the effect to:

- increased insulin secretion;
- reduced intestinal glucose absorption;
- α -amylase/ α -glucosidase inhibition;
- antioxidant activity;
- β -cell protection;
- improved mitochondrial function.

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However, none of these mechanisms were measured in this experiment.

The manuscript should clearly identify these statements as **proposed mechanisms based on previous literature**, not mechanisms established by the present study.

12. Introduction is excessively long

The Introduction contains a large amount of general information on:

- diabetes;
- oxidative stress;
- individual medicinal plants;
- herbal medicine;
- OGTT;
- animal models.

Much of this could be substantially shortened.

The Introduction should focus more directly on:

1. Tablet Dolabi;
2. its composition;
3. existing evidence;
4. knowledge gap;
5. rationale for OGTT;
6. specific research hypothesis/objective.

A more concise Introduction would improve the manuscript considerably.

13. Literature review needs updating

Although many references are relevant, several are relatively old.

The authors should incorporate **recent literature**, particularly concerning:

- polyherbal antidiabetic formulations;
- *Gymnema sylvestre*;
- Unani pharmacology;
- experimental OGTT methodology;
- herbal-product standardization;
- animal-study reporting standards;
- safety and quality control of traditional medicines.

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14. Animal-study reporting standards should be followed

The manuscript should be revised according to appropriate animal research reporting principles, particularly **ARRIVE guidelines**.

Important information should include:

- animal characteristics;
- sample-size determination;
- inclusion/exclusion criteria;
- randomization;
- blinding;
- experimental procedures;
- adverse events;
- statistical methods;
- individual animal data where possible.

15. Results section needs improvement

The Results section is currently too descriptive.

The authors should provide:

- complete statistical comparisons;
- exact p-values;
- AUC/iAUC;
- graphical representation with error bars;
- individual data points if possible;
- clear statistical annotations.

The phrase "**Table X**" should be corrected to the actual table number.

"Graph 3.1" should preferably be presented as a properly numbered **Figure** with an informative legend.

16. Table presentation requires revision

The table should clearly identify:

- n;
- units;
- statistical test;
- comparison group;
- significance level.

For example, the footnote should specify exactly what the superscript means.

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Also, "SE" should be written consistently as **SEM** if standard error of the mean is intended.

17. Important missing physiological/experimental details

The authors should clarify:

- fasting duration for each group;
- whether water remained available during fasting;
- exact age and body weight distribution;
- sex distribution;
- route and volume of administration;
- time between treatment and glucose challenge;
- glucometer model/manufacturer;
- blood sampling method;
- whether measurements were performed in duplicate;
- calibration/quality-control procedure for glucometer;
- whether animals were restrained and how stress was minimized.

18. Significance of the study

The study has **preliminary significance** because it provides an initial experimental assessment of a specific Unani polyherbal formulation rather than examining only one medicinal plant.

If the findings are reproducible, the work could serve as a preliminary basis for:

- diabetic animal-model studies;
- formulation standardization;
- pharmacokinetic investigations;
- mechanism-of-action studies;
- toxicity studies;
- eventual clinical research.

However, the current study should **not be presented as establishing clinical efficacy or therapeutic effectiveness in diabetes**.

19. Key points that should be addressed before publication

Essential revisions

1. Provide the **IAEC approval number/date**.
2. Provide details of **sample-size determination**.
3. Clarify male/female animal distribution.
4. Explain randomization and blinding.
5. Specify the exact treatment-to-glucose interval.

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6. Clearly identify the vehicle actually used.
7. Provide complete product/manufacturer/batch information for Tablet Dolabi.
8. Provide details and results of the acute toxicity study.
9. Reconsider the statistical analysis because measurements are repeated within animals.
10. Report exact p-values.
11. Calculate and report **OGTT AUC/iAUC**.
12. Recheck the percentage-reduction methodology.
13. Remove or qualify unsupported claims of **synergy**.
14. Moderate claims comparing Tablet Dolabi with glibenclamide.
15. Clearly state that the study was performed in **healthy mice**.
16. Avoid extrapolating the findings directly to diabetic patients.
17. Strengthen the Discussion with recent literature.
18. Revise the Results and figures for better statistical presentation.
19. Follow appropriate **animal research reporting guidelines**.
20. Revise the Conclusion so that it reflects the preliminary nature of the evidence.

20. Minor points / editorial corrections

There are numerous formatting and language issues that should be corrected.

Examples include:

- "Bambusabambos" → **Bambusa bambos**
- "Gymnemasylvestre" → **Gymnema sylvestre**
- "IncludingRumex" → **Including Rumex**
- "ofGymnema" → **of Gymnema**
- "arepresents $p < 0.001$ compared" → appropriate spacing and formatting.
- Use consistent **mg/kg**, not alternating forms.
- Use **Results**, rather than "Result".
- Use consistent terminology: "blood glucose level/concentration."
- Ensure all cited references actually support the specific claims made.
- Check all botanical nomenclature and authorities.
- Verify all DOI numbers and bibliographic details.
- Ensure references are genuinely formatted according to APA 7th edition.
- Correct table and figure numbering.

21. Recommendation to the Editor

Major Revision

Justification:

The manuscript presents a potentially useful preliminary investigation of Tablet Dolabi, and the inclusion of a glucose-control group, standard drug group, two treatment doses, and multiple OGTT time points are positive features. However, important deficiencies in experimental reporting, statistical methodology,

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interpretation of the OGTT data, formulation standardization, toxicity reporting, and mechanistic claims currently prevent the results from supporting the strength of the conclusions.

In particular, the study's use of **healthy mice rather than a diabetic model**, very small sample size (**n=5/group**), unclear calculation of percentage reduction, absence of AUC/iAUC analysis, inadequate reporting of randomization/blinding, and unsupported claims of synergistic mechanisms require substantial correction.

The study may become suitable for publication after the authors provide the missing methodological information, perform an appropriate statistical reanalysis, strengthen the presentation of the OGTT results, moderate the mechanistic and therapeutic claims, and clearly position the work as a **preliminary acute glucose-tolerance study** rather than definitive evidence of antidiabetic efficacy.

Suggested final reviewer statement

Recommendation: Major Revision. The manuscript addresses a relevant topic and contains potentially useful preliminary findings regarding the effect of Tablet Dolabi on glucose tolerance in Swiss albino mice. Nevertheless, substantial revisions are required concerning experimental design and reporting, statistical analysis, interpretation of percentage glucose reduction, formulation standardization, toxicity assessment, and the strength of the mechanistic and therapeutic conclusions. In particular, the use of healthy animals limits extrapolation to diabetes, and the reported synergistic effects are not directly demonstrated. The authors should undertake a comprehensive revision and provide adequate statistical and methodological justification before the manuscript can be considered further.