Evaluation of Anti-Diabetic Activity of Siddha Drug T. Kusta Gaja Kesari by Alpha Glucosidase and Alpha Amylase Enzyme Inhibition Assay: *In Vitro* Study

Bharathy K.¹*, Dhivya G.¹, Lakshmi Kantham T.¹ and Meena Kumari R.²

¹Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai 47, Tamil Nadu, India
²Department of Gunapadam, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India

*Corresponding Author

Received: 14th September, 2023; Accepted: 25th October, 2023; Published online: 21st November, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.124

**Abstract:** Diabetes mellitus is a metabolic disorder characterized by abnormal levels of metabolism of lipid, protein and carbohydrate leading to poor blood glucose level control. Inhibition of α-glucosidase and α-Amylase decreases glucose release from starch and oligosaccharides, leading to glucose absorption delay and a decrease in postprandial blood glucose levels. The objective of this study was to evaluate the Anti-Diabetic activity of Siddha drug Tablet *Kusta gaja kesari*. The spectrophotometric assay method was used for screening Anti diabetic activity of the siddha formulation Tablet *Kusta gaja kesari* (T.KGK). The drug T.KGK showed encouraging inhibiting capacity of the enzyme alpha amylase with the highest effect of around 53.99 ± 7.854 % and its IC₅₀ is 454 ± 83.71 μg/ml. Highest Inhibiting capacity of the enzyme glucosidase is around 39.6 ± 4.626 % and its IC₅₀ is 649.1 ± 64.39 μg/ml. It is concluded that the test drug T. *Kusta gaja kesari* (KGK) exerts promising Anti-diabetic activity due to successful Alpha glucosidase and Alpha amylase enzyme inhibition.

**Keywords:** Siddha medicine, *Kusta gaja kesari*, Diabetes mellitus, α-glucosidase, α-Amylase inhibitor


https://doi.org/10.33745/ijzi.2023.v09i02.124

This is an Open Access Article licensed under a Creative Commons License: Attribution 4.0 International (CC-BY). It allows unrestricted use of articles in any medium, reproduction and distribution by providing adequate credit to the author(s) and the source of publication.

**Introduction**

Diabetes mellitus is a metabolic disorder characterized by abnormal levels of metabolism of lipid, protein and carbohydrate leading to poor blood glucose level control (Sindhu *et al.*, 2013). American Diabetes Association (ADA) classifies DM into following types: (i) T1DM (Type 1 DM) or juvenile-onset or IDDM (insulin-dependent DM), (ii) T2DM (Type 2 DM) or NIIDM (non-insulin dependent DM); (iii) gestational DM (GDM), which can be diagnosed in women during pregnancy, and (iv) another type of diabetes which was not included in any of the above types (ADASMCD,
Its global prevalence DM in 2000 was calculated to be 2.8%, and is anticipated to increase up to approximately 4.4% in 2030 (WHO, 2016). According to the World Health Organization (WHO), about 415 million people were expected to be affected by diabetes worldwide in 2015, and in 2040 it is anticipated to rise up to 642 million (Kerru et al., 2018).

Inhibition of α-glucosidase and α-Amylase decreases glucose release from starch and oligosaccharides, leading to glucose absorption delay and a decrease in postprandial blood glucose levels (Proenca et al., 2022). Tablet Kusta gaja kesari (T.KGK) consists of Abraga chenduram/parpam (Ash of Mica), kaantha chenduram (Magnetic oxide of Iron), Aya chenduram (Ferum – Iron), Rasa parpam (Ash of Mercury quick silver), savuri pazha saaru (Trichosanthes tricuspidata Lour). It is indicated for the treatment of leprosy, vitiligo, chronic skin diseases (Kuppuswamy and Uthamarayan, 2009). The objective of this study was to evaluate the Anti-Diabetic activity of Siddha drug Tablet: Kusta gaja kesari.

**Materials and Methods**

**Procurement of test drug:**

The test drug was purchased from GMP certified company IMPCOPS (Indian Medical Practitioners Co-operative Pharmacy and Stores). It was then used for in vitro analysis.

**Ingredients of T.Kusta gaja kesari (KGK):**

Ingredients of T.KGK are as follow:

(i) **Abraga chenduram/parpam** (Ash of Mica) (Thiyagarajan, 2004)

(ii) **Kaantha chenduram** (Magnetic oxide of Iron) (Thiyagarajan, 2004)

(iii) **Aya chenduram** (Ferum – Iron) (Thiyagarajan, 2004)

(iv) **Rasa parpam** (Ash of Mercury quick silver) (Thiyagarajan, 2004)

(v) **Savuri pazha saaru** (Trichosanthes tricuspidata Lour)

**Inhibition of Alpha Amylase – In vitro Study (Kumar et al., 2011):**

The α-amylase enzyme (0.5 U/ml) was made by blending 3.24 mg of α-amylase in 100 ml of phosphate buffer (pH 6.9). Serial dilution of the test Sample (KGK) was produced using DD water in the concentrations ranging from 100, 200, 300, 400 and 500 µg/ml. Reference standard used was acarbose 100 µg/ml. 600 µl test sample was mixed to 30 µl of α-amylase enzyme solution and incubated for 15 min at 37°C. To this, 370 µl of substrate, 2-Chloro-4-Nitrophenyl-α-Maltotrioside (CNPG₃) 0.5 mg/ml was put in and incubated for 10 min at 37°C. At last, absorbance was calculated against blank by spectrophotometer at 405 nm. In the absence of the test sample, a control reaction was done.

\[
\% \text{ inhibition} = \left(1 - \frac{\text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \right) \times 100
\]

**Inhibition of α-Glucosidase Enzyme – In vitro Study (Deutschlander et al., 2009):**

DD water was used for preparing test Sample (KGK) in the serial dilution of the 100, 200, 300, 400 and 500 µg/ml concentrations. 20 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) was prepared by diluting 603 mg PNPG in 100 ml of PBS. The α-glucosidase enzyme solution was prepared by liquifying 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) having 20 mg bovine serum albumin. About 10 µl of the test sample at different concentration along with acarbose 100 µg/ml (as a reference standard) was mixed with 250 µl of 20 mM p-nitrophenyl-α-D-glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated for 5 min at 37°C and the reaction started by addition of 250 µl of the α-glucosidase enzyme solution prepared by 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin, after which it was incubated at 37°C for exactly 15 min. Phosphate buffer (250 µl) was included in the place of enzyme for blank. The reaction was then stopped by addition of 1000 µl of 200 mM Na₂CO₃ solution and the amount of p-nitrophenol released was measured by reading the absorbance of
Table 1: Inhibition percentage of Alpha Amylase enzyme for test drug KGK

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Percentage inhibition of KGK</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μg/ml</td>
<td>14.83 ± 5.029</td>
</tr>
<tr>
<td>200 μg/ml</td>
<td>26.99 ± 10.9</td>
</tr>
<tr>
<td>300 μg/ml</td>
<td>38.61 ± 8.258</td>
</tr>
<tr>
<td>400 μg/ml</td>
<td>43.87 ± 7.41</td>
</tr>
<tr>
<td>500 μg/ml</td>
<td>53.99 ± 7.854</td>
</tr>
<tr>
<td>Standard Acarbose</td>
<td>98.52 ± 1.789</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SD (n=3)

Table 2: IC₅₀ Values - KGK and STD for Alpha Amylase Enzyme inhibition

<table>
<thead>
<tr>
<th>Standard / Test Drug</th>
<th>IC₅₀ Value of Alpha Amylase enzyme inhibition ± SD (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGK</td>
<td>454 ± 83.71</td>
</tr>
<tr>
<td>Standard- Acarbose</td>
<td>34.44 ± 12.27</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SD (n=3)

![Graph showing inhibition percentage of test drug KGK and Standard](image)

Fig. 1: Inhibition percentage of test drug KGK and Standard on inhibition of Alpha Amylase Enzyme assay.

Results and Discussion

The drug T.KGK showed encouraging inhibiting capacity of the enzyme alpha amylase with the highest effect of around 53.99 ± 7.854 % (Table 1; Fig. 1) and its IC₅₀ is 454 ± 83.71 μg /ml (Table 2). Standard acarbose showed pronounced inhibition in alpha glucosidase enzyme with the highest inhibition value of around 98.52 ± 1.789 % (Table 1), its IC₅₀ is 34.44 ± 12.27 μg/ml (Table 2). It was observed from the results that the formulation KGK has the highest Inhibiting capacity of the enzyme glucosidase of about 39.6 ± 4.626 % (Table 3; Fig. 2) and its IC₅₀ is 649.1 ± 64.39 μg/ml (Table 4). Standard acarbose showed pronounced inhibition in alpha amylase enzyme activity with the highest inhibition of about 99.12 ± 0.3885 % (Table 3) and its IC₅₀ 22.89 ± 13.39 μg/ml (Table 4).

Among all diabetes cases, 90% of cases falls under T2DM type. It is characterized by beta-cell failure, chronic metabolic imbalance and resistance to insulin. It can be controlled by modifying the way of living through regular sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.
Table 3: Inhibition percentage of test drug KGK and STD on inhibition of α-Glucosidase enzyme study

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Percentage Inhibition of KGK</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μg/ml</td>
<td>9.738 ± 3.859</td>
</tr>
<tr>
<td>200 μg/ml</td>
<td>19.8 ± 2.381</td>
</tr>
<tr>
<td>300 μg/ml</td>
<td>25.48 ± 2.063</td>
</tr>
<tr>
<td>400 μg/ml</td>
<td>31.54 ± 3.222</td>
</tr>
<tr>
<td>500 μg/ml</td>
<td>39.6 ± 4.626</td>
</tr>
<tr>
<td>Standard- Acarbose</td>
<td>99.12 ± 0.3885</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SD (n=3)

Table 4: IC\textsubscript{50} Values - KGK and STD for α-Glucosidase enzyme inhibition assay

<table>
<thead>
<tr>
<th>Standard /Test Drug</th>
<th>IC\textsubscript{50} Value of α-Glucosidase enzyme inhibition ± SD (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGK</td>
<td>649.1 ± 64.39</td>
</tr>
<tr>
<td>Standard- Acarbose</td>
<td>22.89 ± 13.39</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SD (n=3)

Fig. 2: Inhibition percentage of test drug KGK and Standard on inhibition of Alpha Glucosidase enzyme assay.

exercise, dietary changes (WHO, 2016). Factors like environmental, behavioural and genetic paves way to deficiency and resistance to insulin. Global prevalence of T2DM is increasing day by day which makes it a major health issue for public (Riyaphan et al., 2021). The clinical diagnosis of T2DM is done through evaluation of glucose level in plasma-- (i) fasting plasma glucose (>126 mg/dl); (ii) random plasma glucose (>200 mg/dl) (iii) oral glucose tolerance test (>200 mg/dl) (WHO, 2016); or (iv) HbA1C level >6.5% (Chaudhary et al., 2017). A human subject is considered as prediabetic when fasting glucose is more than the normal level but below the threshold i.e., 110–126 mg/dl. They are more prone to neurological pathologies and
cardiovascular diseases and predispose to insulin resistance and diabetes (ADASMCD, 2016). T2DM medicines which are available today cause various adverse events like urinary tract infection, digestive system disorder, nerves, kidneys, and eye damage, high risk of cardiac failure (Hegde et al., 2014).

*Aya chenduram* which is one of the ingredients of *T.Kusta gaja kesari* (KGK) is having hypoglycemic activity (Shanthi, 2008). *Abraga chenduram, Kaantha chenduram, Rasa parpam* is indicated for diabetes in siddha literature (Thiyagarajan, 2004). Hence, the *T.Kusta gaja kesari* (KGK) proves to be a good Anti-diabetic drug.

**Conclusion**

It can be concluded the test drug *T. Kusta gaja kesari* (KGK) exerts promising Anti-diabetic activity due to effective inhibition of alpha Glucosidase and alpha Amylase enzymes. Advanced clinical trials have to be conducted to establish its clinical effectiveness among Diabetes cases.

**References**


ADASMCD (American Diabetes Association Standards of Medical Care in Diabetes) (2016) Summary of Revisions. Diabetes Care 39((Suppl. 1)): S4-SS.


Riyaphan J, Pham DC, Leong MK and Weng CF. (2021) In silico approaches to identify polyphenol compounds as α-glucosidase and α-amylase inhibitors against type-ii diabetes. Biomolecules 11(12): 1877.


