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

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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 IC_{50} is 454 ± 83.71 µg/ml. Highest Inhibiting capacity of the enzyme glucosidase is around 39.6 ± 4.626 % and its IC_{50} 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

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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,

2010). Its global prevalence DM in 2000 was calculated to be 2.8%, and is anticipated to increase upto approximately 4.4% in 2030 (WHO, 2016). According to World Health Organization (WHO), about 415 million people was 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 ($CNPG_3$- 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.

$$\% \text{ inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

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 Na_2CO_3 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

Concentration (µg/ml)	Percentage inhibition of KGK
100 µg/ml	14.83 ± 5.029
200 µg/ml	26.99 ± 10.9
300 µg/ml	38.61 ± 8.258
400 µg/ml	43.87 ± 7.41
500 µg/ml	53.99 ± 7.854
Standard Acarbose	98.52 ± 1.789

Each value represents Mean ± SD (n=3)

Table 2: IC_{50} Values - KGK and STD for Alpha Amylase Enzyme inhibition

Standard / Test Drug	IC_{50} Value of Alpha Amylase enzyme inhibition ± SD (µg/ml)
KGK	454 ± 83.71
Standard- Acarbose	34.44 ± 12.27

Each value represents Mean ± SD (n=3)

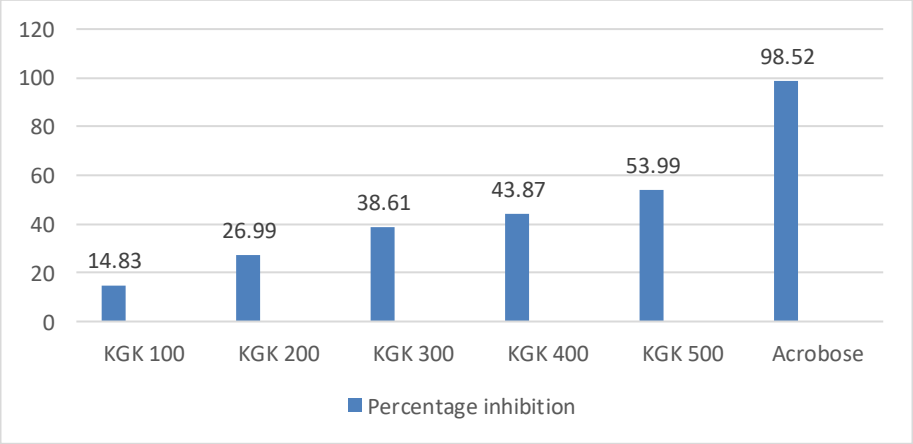

Fig. 1: Inhibition percentage of test drug KGK and Standard on inhibition of Alpha Amylase Enzyme assay.

sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.

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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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

Table 3: Inhibition percentage of test drug KGK and STD on inhibition of α-Glucosidase enzyme study

Concentration (µg/ml)	Percentage Inhibition of KGK
100 µg/ml	9.738 ± 3.859
200 µg/ml	19.8 ± 2.381
300 µg/ml	25.48 ± 2.063
400 µg/ml	31.54 ± 3.222
500 µg/ml	39.6 ± 4.626
Standard- Acarbose	99.12 ± 0.3885

Each value represents Mean ± SD (n=3)

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

Standard /Test Drug	IC_{50} Value of α-Glucosidase enzyme inhibition ± SD (µg/ml)
KGK	649.1 ± 64.39
Standard- Acarbose	22.89 ± 13.39

Each value represents Mean ± SD (n=3)

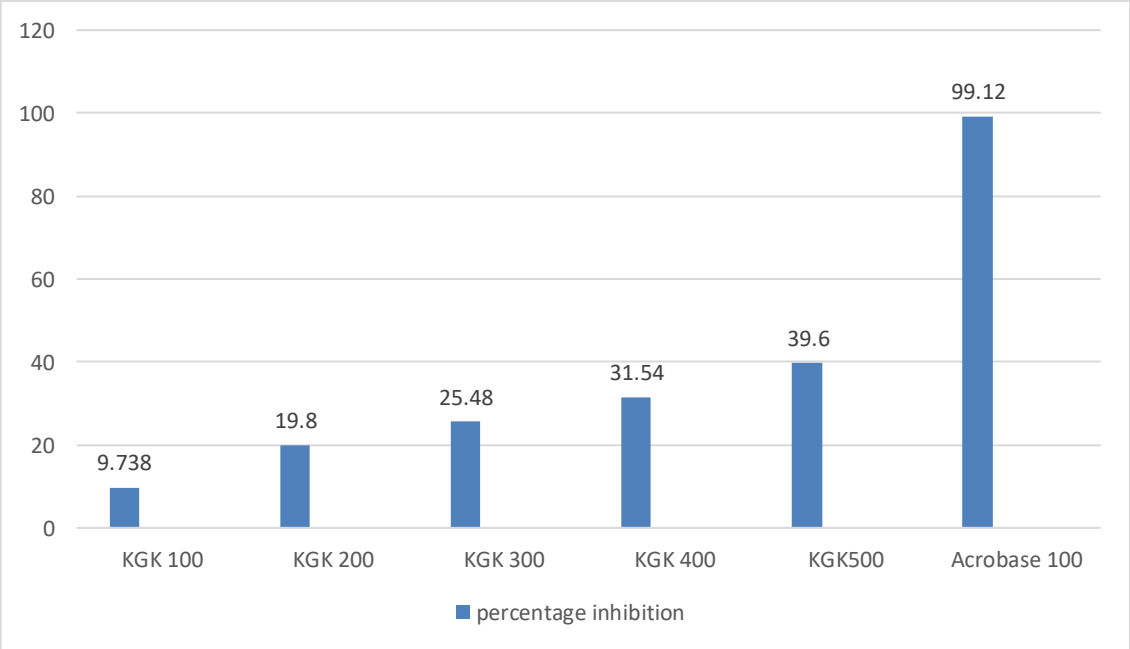

Fig. 2: Inhibition percentage of test drug KGK and Standard on inhibition of Alpha Glucosidase enzyme assay.

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 causes 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.

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