Title: Anti-diabetic and Anti-hyperglycaemic effect of *Kaempferia Pulchra* extracts on Streptozotocin Induced Diabetic Rats

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Abstract: Diabetes mellitus (DM) is a multifaceted metabolic disorder. The aim of the study was to investigate the role of anti-diabetic and anti-hyperglycaemic activity of *Kaempferia pulchra* ethyl acetate fraction from the rhizome on streptozotocin induced diabetic rats. Completely Randomized Design was used to evaluate the anti- diabetic activity of different doses as 250 mg/Kg (G3), 500 mg/Kg (G4), 1000 mg/Kg (G5) of *Kaempferia pulchra* ethyl acetate fraction with Glibenclamide 25mg/kg (G6) as standard was dosed orally and evaluated with diabetic and normal rats for 28 days. The parameters observed were glucose levels in rat blood. The biochemical parameters such as serum SGOT, SGPT, total Proteins, triglyceride, Cholesterol, were analyzed for knowing the anti-diabetic activity of *Kaempferia pulchra* extract which shows reduction in blood glucose dose dependently in comparision with diabetic group by proving anti-diabetic effect and also significantly ($p<0.05$) reduced the biochemical parameters compared to diabetic group.

Key words: Diabetes, Glibenclamide, Hyperglycemia, *Kaempferia pulchra*

Introduction

Diabetic mellitus is a serious metabolic disorder, approximately 422 million people affected worldwide. Also described as chronic hyperglycemia, resistance to insulin and/or caused by functional failure of β-pancreatic cells showing secondary insulin deficiency (Association, 2017 and Chen et al., 2017). In the world India is the second large diabetic country, with approximate of around 70 million of diabetic adults. By 2045 can expect to rise up to 135 million (Krug 2016). Due to type-2 diabetes is the reason for increased pateents (>90%), can be due to functional deficiency of pancreas or increased resistance to insulin relatively, leads to glucose tolerance impairment (Bupesh et al., 2022). In addition evidence scientifically proposed that postprandial hyperglycemia effectively stimulates dysfunction of endothelium during diabetes, also for the expansion of various pathological problems like retinopathy, nephropathy, cardiovascular disease, atherosclerosis and neuropathies are the oxidative stress and inflammatory reactions are the responsible factors (Giri et al., 2018). Recently from the active parts of the many medicinal plants, directly or indirectly there is half of all pharmaceutical medication have been generated and bring them at position in research initiatives (Saravanan et al., 2022 a and b). approximately 800 plants have medicinal benefits to reduce problems with diabetes and associated problems according to ethnobotonical information (Pothiaraj et al., 2022; Sivaraman and Khanna 2016).many studies have recorded various plant-derived active constituents like polysaccharides, galactomannan, glycopeptides, glycosides, terpenoids, steroids, carbohydrates inorganic ions and amino acids which alter metabolic cascades, which affect the body's blood glucose level (Balagi et al., 2022 and Hashiguchi et al., 2022). Apart from the their mimetic properties of insulin some of reports also propose that those bioactive principles also influence the secretion of insulin by pancreas. Kaempferia is belongs to Zingiberaceae having vast ethno medicinal importance important species is Kaempferia pulchra Ridl is the important species, which is greatly known to the area of tropical Southeast Asian countries; But in Indian peninsula it lacks the same status (Vincent et al., 1992; Musa et al., 2011; Singh et al., 2023). In traditional medicine the K. pulchra is extensively used to treat fever, stomach issues, urinary infections and coughs. In addition this also relieving from heat, carminative. Blood-stimulating and diuretic properties (Raina et al., 2015 and Labrooy et al., 2018). Also terpenoids from K. pulchra rhizomes are evidenced with anticancer, anti-mutagenic, anti-inflammatory and antimicrobial properties (Vijayaraghavan et al., 2012 and Kanaiyalal et al., 2023). Pancreatic injury is induced by streptozotocin is generally used as animal model of type-1

diabetes mellitus (TIDM) and commonly presents common characteristics to those found in diabetic nephropathy in human. Originally STZ is identified as a antibiotic, and is a congener of N acetyl glucosamine, which is transported into β-cells of pancreas by GLUT-2 and it is the source for β-cell toxicity. Which leads to deficiency of insulin (Tesch and Allen, 2007). In the present study, Kaempferia pulchra at different concentrations were evaluated for knowing the anti-diabetic and anti-hyperglycaemic by measuring glucose levels, oxidative stress, lipid levels, and glucose metabolism.

Materials and methods

Plant collection and authentication of plant

From different areas of Thiruvananthapuram district of Kerala, India the plant was collected from April to September (8.17°N 76.41°E) due to plant will die off in winter. And was authenticated at University of Nagaland, Department of Forest Science Lumami, Zunheboto, Nagaland. Specimens were stored in the centre of University for Biotechnology greenhouse (NUFS/2021/1004).

Preparation of extract

K. pulchra fresh leaves and rhizomes were collected and washed using distilled water, made into several pieces and subjected to air dried and powdered. Dried powder was filled into Soxhlet apparatus for extraction for 24 hours with water as solvent. (Himanshu et al., 2023)

Protocol approval

Study protocol was approved by the Institutional Animal Ethics Committee (IAEC) IAEC clear 31/21 dated 2 Sep 2022.

Wistar Albino male rats (180-200 g) were procured from registered breeder, maintained 12h light and dark cycle, experimental animals were acclimatized for 7 days before initiation of the study, randomized based on body weight. Animals were housed in sterile polypropylene cages.

Acute toxicity study

Acute oral toxicity study was carried out at different doses was performed. The rats were fasted overnight and were treated at doses of 2000 and 5000 mg/ Kg b.w extract orally. Animals were observed for 48 hours for clinical signs like and any signs of neurological/ behavioral changes,

convulsions, coma or death were recorded. an observation period of 14 days, the procedure was carried out as per OECD 423 (Kaushik et al., 2009).

Induction of Diabetes in experimental animals

Experimental animals were kept fasted for 12 hours and administered through i.p. with freshly prepared Steptozotocin (STZ) (Himedia) at concentration of 55mg/kg with cold citrate buffer (Ph 4.5), After the treatment with STZ, animals were allowed overnight to drink 5% glucose solution to avoid hypoglycaemia induced by drug (U. G. Icoz et. al., 2017).

On the third day after the treatment with STZ injection, rats with persistent hyperglycaemia i.e. > 250 mg/dl, the fasting blood glucose were considered as Diabetic and same animals were used for further experiments.

***In vivo* Anti diabetic study**

The ethyl acetate fraction from the rhizome extract (TRF) used as test sample

The experimental animals were divided into 6 groups, each group consists of eight animals (n=8) as per study design (U G. Icoz ct. a1., 2017) and dosing was done orally for 28 days. Group 1 serves as Vehicle Control, Group 2 serves as Diabetic Control, Group 3 as TRF 250mg/kg body weight), Group 4 (TRF 500 mg/kg body weight), Group 5 (TRF 1000 mg/kg body weight) and Group 6 serves as Reference Standard (Glibenclamide, 25 mg/kg body weight).

Completion of 28 days treatment, rats were humanely euthanized and blood collection as well as relevant organ collection like pancreas and liver was performed. The collected organs were weighed and stored in 10% formalin (S. K. Hassan ct. at., 2015).

Estimation of biochemical parameters

The serum Biochemical parameters like SGOT, SGPT, protein, urea, triglycerides, cholesterol, ALP, serum glucose, plasma insulin, serum lipid profile were analyzed by Autoanalyser (XL 640 - Erba India) standard procedure (S. K. 1-lassan et. al. 2015).

Sl.no	**Dosage details**
T1	Normal control
T 2	Diabetic Control
T 3	Diabetic + *K.pulchra* 250 mg/kg

Treatment details	T 4	Diabetic + *K. pulchra* 500 mg/kg
	T5	Diabetic + *K. pulchra* 1000 mg/kg
	T6	Diabetic + Glibenclamide 25 mg/kg

Fasting blood glucose in each group was measured by blood glucose meter at the beginning of the experiment and at the same time every week (blood was collected at day 7, 14, 21, 28, 35, and 42), while the bodyweight was recorded every 48 h. At the end of the sixth week, the rats were sacrificed by isoflurane and the orbital blood was collected in capillary tubes. The light-yellow supernatant from the orbital blood was collected after centrifugation at 2500 rpm for 20 min. The liver and kidney tissues were homogenized with a biospecimen homogenizer under ice-cold saline solution, and the supernatant was collected by centrifugation at 3000 rpm for 20 min and used for subsequent analysis.

Results and discussion

The effect of *Kaempferia pulchra* extract on blood glucose level in diabetic mice is presented in **Table 1** and data shows that from day zero to day 29, blood glucose level increased in diabetic group(at day 0 was found 289 mg/dl and at day 29 was 422 mg/dl) in comparison with normal control group(at day 0 was found 94.6 mg/dl and at day 29 was 96.2 mg/dl). Whereas the *Kaempferia pulchra* extract shows dose dependent action at 250 mg/kg was found to reduce 229.5 mg/dl, at 500 mg/kg was found 178.3 mg/dl, at 1000 mg/kg was 128.4 mg/kg at day 29 in comparision with diabetic control group and extract action was almost equal to standard drug i.e. at 25 mg/kg was 119.6 mg/dl. And the same is presented graphically in **Figure 1.** The effect of *Kaempferia pulchra* extract on biochemical parameters in serum were shown statistical significant results dose dependently comparative to diabetic inducer group presented in **Table 2**. HDL was increased with extract and at 1000mg/kg was found 40.00±1.155 mg/dl compared to diabetic group i.e. 35.30±1.217 mg/dl and standard shows 40.83±2.028. Total protein was decreased with extract i.e. at 1000 mg/dl was found 83.27±1.213 mg/dl compared to diabetic group i.e. 138.5±0.000 mg/dl and standard decreased upto 84.30±1.400mg/dl. SGOT was decreased at

1000mg/kg was 60.33±0.8819 mg/dl relative to diabetic group shows 120.7±0.9333 mg/dl and standard was 67.97± 0.8192. SGPT was found decreased to 60.33±0.8819mg/dl at 1000mg/kg of extract relative to diabetic group i.e. 116.3±0.8819 mg/dl and standard shows 58.67±0.8819mg/dl. Cholesterol was also decreased at 1000 mg/kg was 123.4±2.621 mg/dl compared to diabetic group which was 214±2.646 mg/dl and standard was 114.7±2.578 mg/dl. Triglyceride was found at 1000mg/kg i.e. 81.73±2.322mg/dl relative to diabetic group was 147.1±3.355mg/dl and standard was found 79.67±0.7333mg/dl data were represented graphically in **Figure 2**.

Table 1: Effect of *Kaempferia pulchra* extract on blood glucose level on different days as shown below.

Groups	Day 0	Day 3	Day 7	Day 14	Day 21	Day 29
Normal Control	94.6	94.33	95	95.9	96	96.2
Diabetic Control	289	309	365	412	418	422
Diabetic + *K.pulchra* 250 mg/kg	273.1	301.1	281.2	261.2	237.5	229.5
Diabetic + *K. pulchra* 500 mg/kg	281.4	303.2	263.2	227.2	187.1	178.3
Diabetic + *K. pulchra* 1000 mg/kg	285.4	305	238	175	135	128.4
Diabetic + Glibenclamide 25 mg/kg	277.2	303	215	161	125	119.6

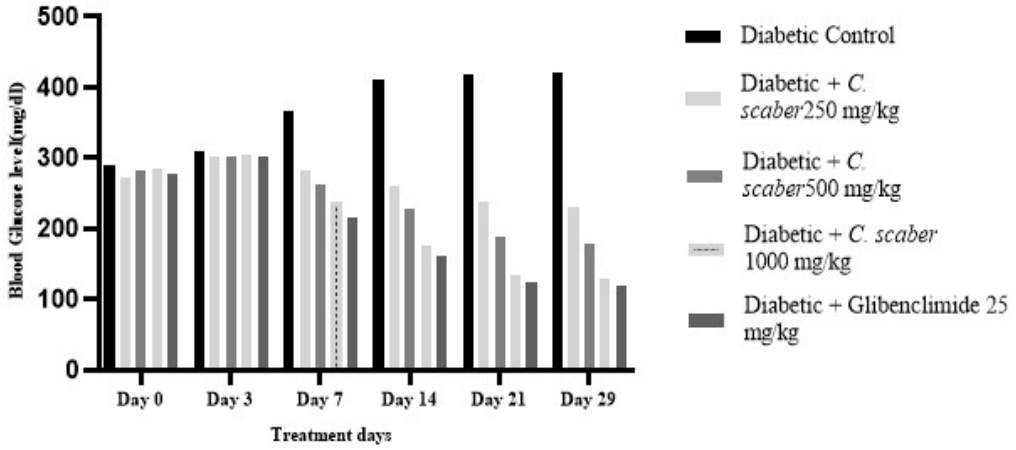


Figure1: Effect of *Kaempferia pulchra* on blood glucose level at different day's time interval. Results showed statistically significant reducedin comparision with diabetic control group.

Table 2: Effect of *Kaempferia pulchra* extract on biochemical parameters in rat serum as shown below. Data were expressed as Mean±SEM.

Groups	HDL	Proteins	SGOT	SGPT	Cholesterol	Triglyceride
Diabetic Control	35.30± 1.217	138.5± 0.000	120.7± 0.9333	116.3± 0.8819	214± 2.646	147.1± 3.355
Diabetic + *K.pulchra* 250 mg/kg	40.00± 1.155	93.77± 0.8819	73.97± 1.157	71.60± 0.8327	138.4± 1.705	91.23± 2.373
Diabetic + *K. pulchra* 500 mg/kg	41.30± 1.069	85.07± 1.453	72.93± 0.7688	68.53± 0.5812	125± 2.318	83.43± 2.399
Diabetic + *K. pulchra* 1000 mg/kg	41.60± 1.155	83.27± 1.213	69.27± 0.9955	60.33± 0.8819	123.4± 2.621	81.73± 2.322
Diabetic + Glibenclamide 25 mg/kg	40.83± 2.028	84.30± 1.400	67.97± 0.8192	58.67± 0.8819	114.7± 2.578	79.67± 0.7333

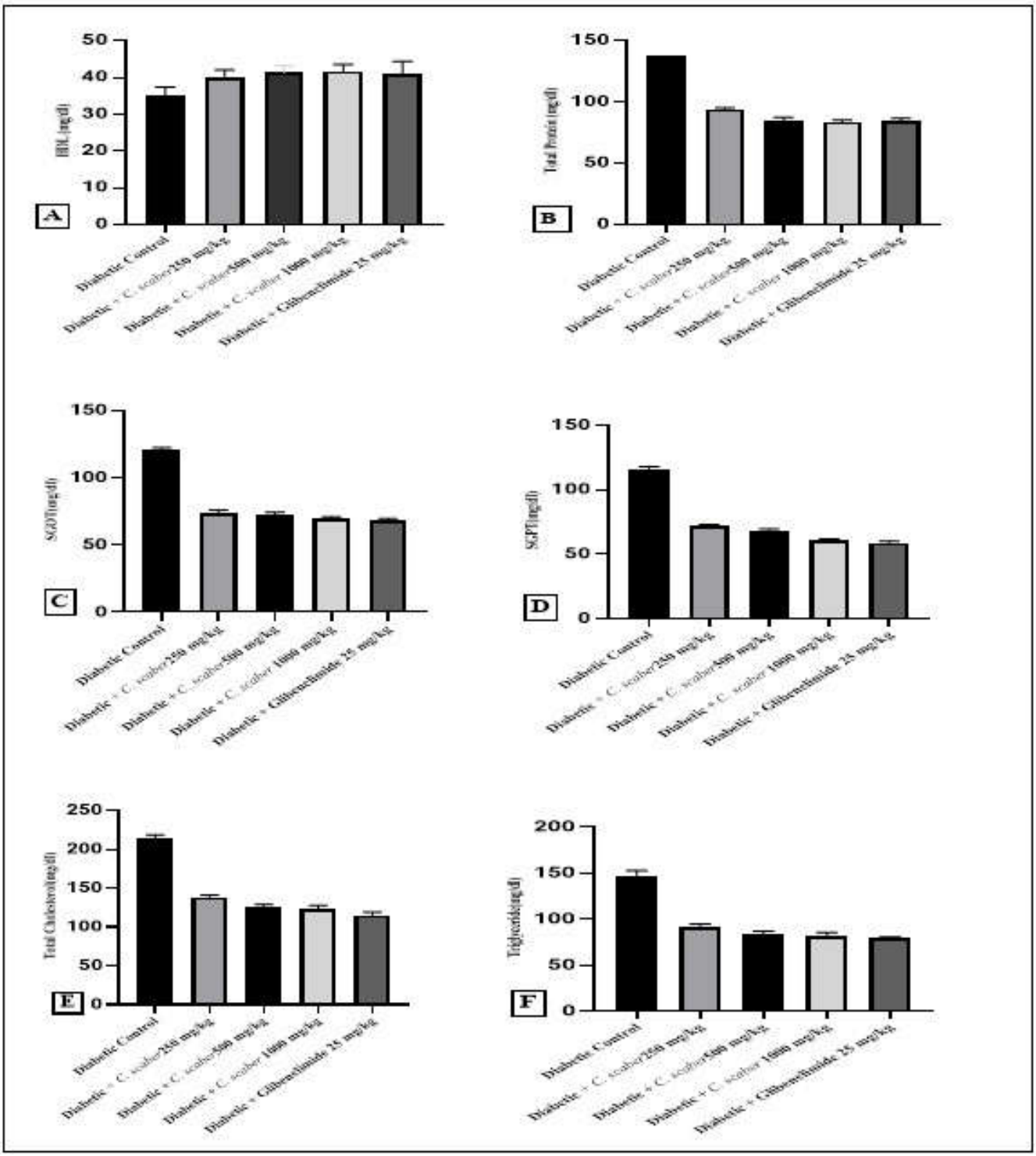


Figure 2: The effect of *Kaempferia pulchra* extract on serum biochemical parameters as follows **A.** HDL, **B.** Total proteins, **C.** SGOT, **D.** SGPT, **E.** Total cholesterol, **F.** Triglycerides shows statistically significant reduction ($p<0.05$) with comparison of diabetic group.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Graphpad prism 8.0.2(263) was used for experimental data processing. Analysis of variance (ANOVA) was employed separating means at $p < 0.05$ using the post hoc tests.

Conclusion

Based on the obtained results *Kaempferia pulchra* extract may possess anti-diabetic activity and anti-hyperglycemic effect in experimental animals. Further clinical studies will be required to confirm the effectiveness of the above plant extract.

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Ethical statement

"Not applicable".

Conflict of interest - Disclose

Authors have no conflict of interest

Authors' contribution

"Conceptualization: Dr.Kalpana Ashish Dabhade and Himanshu Kanaiyalal Solanki; Data collection: Himanshu Kanaiyalal Solanki; Data analysis: Dr.Kalpana Ashish Dabhade and Himanshu Kanaiyalal Solanki; Figure preparation: Dr.Kalpana Ashish Dabhade and Himanshu Kanaiyalal Solanki All authors critically reviewed the manuscript and agreed to submit final version of the manuscript."

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