Research Article

Antihyperlipidemic effect of *Kaempferia galanga* in streptozotocin-induced diabetic rats

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ABSTRACT

Background: Diabetes mellitus is one of the worldwide increasing problems characterized by metabolic deregulation. One of the major factors contributing to cardiovascular risk in patients with Type 2 diabetes is abnormalities of lipoprotein metabolism. Due to various side effects caused by available drugs, the search for new antidiabetic and antihyperlipidemic drugs from herbal plants has become essential. **Aim:** The present study was carried out to evaluate the antihyperlipidemic potential of hydroethanolic extract of *Kaempferia galanga* rhizome in streptozotocin (STZ)-induced diabetic rats. **Materials and Methods:** Male albino Wistar rats (6 weeks old), weighing 170–200 g, were used for the present study. Diabetes was induced by intraperitoneal injection of STZ at a dose of 60 mg/kg body weight (bw). Oral administration of hydroethanolic extract of *K. galanga* rhizome at 250 mg/kg bw and 500 mg/kg bw was carried out for 28 days. Blood was collected by cardiac puncture and the serum was separated and analyzed for biochemical parameters. **Results:** The reports showed a significant reduction in the levels of protein, total cholesterol, phospholipids, triglyceride, very low-density lipoproteins (LDL), and LDL compared to the diabetic group which was reverted to near-normal levels in treated groups. The level of high-density lipoprotein cholesterol significantly increased in treated groups with the improvement of diabetic dyslipidemia. **Conclusion:** The present findings infer that the rhizome extract is capable of controlling hyperlipidemia. Further studies can be carried out to isolate the active compound for the management of diabetes-induced complications.

KEY WORDS: Cholesterol, Diabetes, Hyperlipidemia, *Kaempferia galanga*, Lipid level, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM), a chronic a metabolic disorder, is characterized by elevated levels of blood glucose resulting from an abnormality in insulin production, insulin action, or both.[1] It is one of the worldwide increasing problems associated with peripheral vascular disease, nephropathy, neuropathy, and retinopathy.[2] India is one of the epicenters of the global DM pandemic. Demographic changes and rapid socioeconomic development, along with increased susceptibility for Indian individuals, have increased by DM in India.[3] Type 1 DM, also referred to as autoimmune diabetes, occurs due to pancreatic beta-cell loss leading to insulin deficiency and thereby leads to hyperglycemia.[4] Type 2 DM (T2DM) is characterized by pathophysiologic abnormalities, namely, muscle insulin resistance, hepatic insulin resistance, progressive beta-cell failure, and apoptosis.[5] Patients with T2DM experienced high risk of cardiovascular disease and cardiovascular mortality relative to healthy individual. A major contributor to the increased cardiovascular risk linked with Type 2 diabetes is dyslipidemia, which encompasses abnormalities in all lipoproteins.[6]

New drug discoveries rely on natural products, such as plant extract, either as isolated compounds or as standardized extracts, due to matchless availability of chemical diversity.[7] Traditional plant remedies or herbal formulations exist and are still widely used since ancient times. Many plants and plant-derived compounds have been used in the management of diabetes; thus, they provide a potential source of hypoglycemic drugs.[8] The family Zingiberaceae is highly known for its medicinal property which was found to be distributed widely in Southeast Asia. The ginger family includes 53 genera and over 1200 species. About 20 genera and around more than 200 species exist in India and seemed to be one of the richest and varied regions for Zingiberaceae.[9] *Kaempferia galanga* L. (Family: Zingiberaceae) is a

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Website: jprsolutions.info | ISSN: 0975-7619

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Received on: 07-10-2019; Revised on: 09-11-2019; Accepted on: 14-12-2019

rare medicinal plant with effective medicinal activities. The leaves, rhizome, and root tubers of the plant own several medicinal applications. It is composed of 60 species of small rhizomatous herb and is used for the treatment of various ailments such as abdominal pain, muscular swelling, hypertension, rheumatism, ascariasis, and tumor asthma.[10] With this background, the present investigation was carried out to evaluate antihyperlipidemic potential of hydroethanolic extract of *K. galanga* rhizome in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Collection of Plant Material

K. galanga rhizome was used for the present study. It was collected from I-AIM (FRLHT), Bengaluru. The genus and species were identified and certified by the Botanical Survey of India, Tamil Nadu Agriculture University campus, Coimbatore, Tamil Nadu, India, with the voucher number BSI/SRC/5/23/2018/Tech/3455.

Preparation of Plant Extract

The rhizome portion of *K. galanga* was used for the present study. It was washed in running tap water, shade dried, and ground to a coarse powder. It was then extracted with 50% hydroethanol in the Soxhlet apparatus. The extract obtained was concentrated, dried, and kept in desiccator. The sample was then used for further treatment.

Procurement of Animals

Male albino Wistar rats (6 weeks old), weighing 170–200 g, were used for the present study. The animals were housed in clean polypropylene cages and retained in a well-aerated temperature-controlled animal house with a constant 12 h light/dark schedule. The animals were fed with standard rat diet and clean drinking water was made available *ad libitum*. All animal procedures were performed after approval from the ethical committee (IAEC No. KMCRET/Ph.D/03/2016-17) and by following the recommendations for the proper care and use of laboratory animals.

Acute Toxicity Study

Acute oral toxicity study was conducted for *K. galanga* as per the Organization for Economic Cooperation and Development guidelines 423.[11] Healthy male albino Wistar rats were kept in fasting overnight and were orally fed with the extract at increasing doses of 100, 500, 1000, and 2000 mg/kg body weight (bw). After the oral administration of hydroethanolic extract *K. galanga,* the animals were observed for behavioral changes and mortality during the first 30 min, 1 h, 2 h, and 6 h and periodically for the first 24 h with special attention given for the first 4 h. The observation was prolonged for 14 days regularly for toxicity determination of *K. galanga.*

Induction of DM in Experimental Animals

The animals were kept overnight fasting and diabetes was induced in overnight fasted rats by intraperitoneal injection of STZ at a dose of 60 mg/kg bw in 0.1 M cold citrate buffer (pH 4.5). To prevent the STZ-induced hypoglycemia, rats received 10% dextrose solution after 6 h of STZ administration for the next 24 h. Induction of diabetes was verified after 72 h by measuring blood glucose level with strips using a glucometer (Accu-Chek® Active, Roche Diagnostic Corporation, Mannheim, Germany) and the animals were allowed for 14 days for the stabilization of blood glucose levels. On day 14, animals exhibiting blood glucose level higher than 250 mg/dL were considered as diabetic and used for the experiments.[12]

Study Design

The experimental rats were divided into six groups, each comprising six animals. The study period was 28 days.

- Group I: Normal healthy control rats administered with saline (0.9% w/v)
- Group II: STZ (60 mg/kg bw) induced diabetic control rats administrated with saline (0.9% w/v)
- Group III: Diabetic rats treated with hydroethanolic extract of *K. galanga* rhizome (250 mg/kg bw)
- Group IV: Diabetic rats treated with hydroethanolic extract of *K. galanga* rhizome (500 mg/kg bw)
- Group V: Diabetic rats treated with glibenclamide (600 µg/kg bw)
- Group VI: Normal healthy rats treated with hydroethanolic extract of *K. galanga* rhizome (500 mg/kg bw).

Collection of Blood Sample from the Experimental Rats

After the end of the treatment period (28 days), the animals were fasted overnight and sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by cardiac puncture and the serum was separated by centrifugation at 2500 rpm for 15 min.

Determination of Biochemical Profile

Serum samples of experimental animals were subjected to various biochemical analysis, namely, serum total protein, cholesterol, triglycerides (TGs), low-density lipoproteins cholesterol (LDL-C), very LDL cholesterol (VLDL-C), and high-density lipoprotein cholesterol (HDL-C), and phospholipids. The serum protein was estimated by the method.[13] Cholesterol present in the serum was determined by adopting the method.[14] The TG was estimated by the method.[15] HDL-C was determined using Diagnostic Kit Qualigens Diagnostics, Mumbai, India.[6] VLDL-C and LDL-C were calculated as VLDL-C = TG/5 and LDL-C = total cholesterol − (HDLC + VLDL-C).[16]

Phospholipids present in the serum were estimated by the method.[17]

Statistical Analysis

The values were expressed as mean ± standard error of the mean. The data of all the parameters were analyzed statistically by one-way analysis of variance followed by Dunnett's test.

RESULTS AND DISCUSSION

Acute Toxicity Studies

Acute toxicity studies showed no discernible behavioral changes on oral administration of hydroethanolic extract of *K. galanga* rhizome up to 2000 mg/kg bw. No mortality was observed at this dose during 72 h observation period.

Estimation of Biochemical Parameters

The levels of protein in the treated and control group observed are depicted in Table 1. The protein level observed in STZ-induced diabetic rats was significantly decreased when compared to that of control rats. The reduced level of serum total protein in diabetic rats may be attributed to increased muscle proteolysis, decreased amino acid uptake, and increased conversion of glycogenic amino acid to carbon dioxide and water, reduction in protein synthesis and absorption.[18] In DM, proteins are spared as an energy source as the body cells are unable to utilize glucose as a source of energy. This led to decreased protein storage which, in turn, reduces bw.[19] Administration of *K. galanga* extract to the diabetic rats restored the level of protein to near-normal level which might be due to increase in amino acid uptake mediated by insulin, anabolism of protein, and/or protein degradation inhibition.[20]

The lipid profile of diabetic and treated animals is shown in Table 2.

In the current study, a higher level of serum cholesterol was observed in diabetic rats [Table 2]. Further, STZ-induced diabetic rats when treated with hydroethanolic extract of *K. galanga* showed significantly reduced levels of serum cholesterol. These findings are correlated with the similar work where an elevation in serum cholesterol was observed in the untreated diabetic group of animals, the animals treated with aqueous extract of *Mangifera indica* exhibited a significant decrease in serum cholesterol.[21]

During diabetes, the activity of lipase enzyme increases lipolysis and releases more free fatty acids in the circulation due to of lack of insulin.[22] Increase in fatty acid concentration, in turn, increases the beta-oxidation of fatty acids by increasing the activity of HMG-CoA reductase for generating more cholesterol. Insulin also increases the receptor-mediated removal of LDL-C and decreased activity of insulin during diabetes causes hypercholesterolemia.[23]

Hypertriglyceridemia and hypercholesterolemia are the most common lipid abnormalities associated with diabetes[24] and it is well known that the level of glycemic control is the major marker of serum level of TG.[25] Several investigators demonstrated that near normalization of the blood glucose level resulted in significant reductions in levels of plasma cholesterol and TG levels. A significant reduction in TG level in the extract of *K. galanga* treated animals was observed, indicating hypolipidemic effect. This observed hypolipidemic effect might be due to decreased cholesterogenesis and fatty acid synthesis.[26] This is as per the report[27] where the administration of Momordica cymbalaria fruit significantly decreased the levels of TG s in alloxan-induced diabetic rats.

The level of VLDL and LDL-C was increased in diabetic rats which then decreased significantly in treated groups. The treated groups exhibited and increased levels of HDL-C than the diabetic control group [Table 2]. The findings demonstrated that the hydroethanolic extract of *K. galanga* has a hypolipidemic effect. The diabetic animals exhibited a significantly increased level of serum phospholipid compared to that of the control group [Table 3]. A marked significant reduction in the phospholipids level was observed in the animals treated with plant extract. Administration of *K. galanga* hydroethanolic extract of 500 mg/kg bw showed a better hypolipidemic effect compared to 250 mg/kg bw. Deficiency of insulin inactivates the lipoprotein lipase promoting liver conversion of free fatty acids into phospholipids and cholesterol which get discharged into the blood resulting in elevated serum phospholipids level.[28] Alcoholic extracts of *Phaseolus vulgaris* significantly reduced the lipid profile in diabetic rats reflecting its

Table 1: Effect of *K. galanga* on serum protein level in experimental rats

Groups	Total protein (g/dl)
I	25.14±0.16
II	17.02±0.34^{a}
III	19.97±0.92^{b}
IV	21.81±0.30^{bc}
V	23.49±0.41^{b}
VI	24.52±0.48

Values are expressed as the mean±standard error of the mean; Statistical significance (*P*) calculated by one-way analysis of variance followed by Dunnett's $^{c}P<0.001$, $^{b}P<0.01$, $^{a}P<0.05$ calculated by comparing treated group with induced group, ^{bc}Significant between the treated groups at $P<0.05$. Group I: Normal healthy control rats administered with saline (0.9% w/v). Group II: Streptozotocin (60 mg/kg bw) induced diabetic control rats administrated with saline (0.9% w/v). Group III: Diabetic rats treated with hydroethanolic extract of *K. galanga* rhizome (250 mg/kg bw). Group IV: Diabetic rats treated with hydroethanolic extract of *K. galanga* rhizome (500 mg/kg bw). Group V: Diabetic rats treated with glibenclamide (600 µg/kg bw). Group VI: Normal healthy rats treated with hydroethanolic extract of *K. galanga* rhizome (500 mg/kg bw). *K. galanga*: *Kaempferia galangal,* bw: Body weight

Table 2: Effect of *K. galanga* on lipid profile in experimental rats

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	High-density lipoprotein cholesterol (mg/dl)	Very low-density lipoprotein cholesterol (mg/dl)	Low-density lipoprotein cholesterol (mg/dl)
I	88.33±3.06	68.57±2.93	49.13±1.94	13.65±3.32	26.25±3.81
II	168.83±1.46[a]	178.67±3.51[a]	36.05±4.50[a]	35.64±5.97[a]	116.17±4.97[a]
III	108.67±2.51[b]	89.06±2.86[b]	39.65±2.45[b]	17.51±4.81[b]	47.65±4.74[b]
IV	96.18±3.04[bc]	79.23±3.31[bc]	46.83±2.74[bc]	15.48±4.26[bc]	32.88±4.47[bc]
V	90.83±3.38[b]	70.88±3.50[b]	47.53±2.70[b]	14.23±2.00[b]	29.70±5.53[b]
VI	89.69±1.59	69.61±1.37	49.71±6.64	13.79±4.64	26.06±4.32

Values are expressed as the mean±standard error of the mean; Statistical significance (*P*) calculated by one-way analysis of variance followed by Dunnett's [c]*P*<0.001, [b]*P*<0.01, [a]*P*<0.05 calculated by comparing treated group with induced group, [bc]Significant between the treated groups at *P*<0.05. Group I: Normal healthy control rats administered with saline (0.9% w/v). Group II: Streptozotocin (60 mg/kg bw) induced diabetic control rats administrated with saline (0.9% w/v). Group III: Diabetic rats treated with hydroethanolic extract of *K. galanga*: rhizome (250 mg/kg bw). Group IV: Diabetic rats treated with hydroethanolic extract of *K. galanga*: rhizome (500 mg/kg bw). Group V: Diabetic rats treated with glibenclamide (600 µg/kg bw). Group VI: Normal healthy rats treated with hydroethanolic extract of *Kaempferia galanga* rhizome (500 mg/kg bw). *K. galanga*: *Kaempferia galangal,* bw: Body weight

Table 3: Effect of *Kaempferia galanga* on phospholipid level in experimental rats

Groups	Phospholipid (mg/dl)
I	86.54±0.79
II	158.89±2.50[a]
III	111.94±4.02[b]
IV	94.89±4.68[bc]
V	90.19±1.06[b]
VI	87.71±2.15

Values are expressed as the mean±standard error of the mean; Statistical significance (*P*) calculated by one-way analysis of variance followed by Dunnett's [c]*P*<0.001, [b]*P*<0.01, [a]*P*<0.05 calculated by comparing treated group with induced group. Group I: Normal healthy control rats administered with saline (0.9% w/v). Group II: Streptozotocin (60 mg/kg bw) induced diabetic control rats administrated with saline (0.9% w/v). Group III: Diabetic rats treated with hydroethanolic extract of *Kaempferia galanga* rhizome (250 mg/kg bw). Group IV: Diabetic rats treated with hydroethanolic extract of *Kaempferia galanga* rhizome (500 mg/kg bw). Group V: Diabetic rats treated with glibenclamide (600 µg/kg bw). Group VI: Normal healthy rats treated with hydroethanolic extract of *Kaempferia galanga* rhizome (500 mg/kg bw). bw: Body weight

hypolipidemic activity and may also decrease the risk of vascular disease and related complications.[29]

CONCLUSION

It is concluded that the protein level observed in STZ-induced diabetic rats was significantly decreased when compared to that of control rats which then reverted to near-normal level on treatment with the extract. A higher level of serum cholesterol in diabetic rats was observed. Further, STZ-induced diabetic rats when treated with hydroethanolic extract of *K. galanga* significantly reduced the serum cholesterol level which may be due to the inhibitory effect of the active principles on enzymes of cholesterol biosynthesis. A significant reduction in TG level in *K. galanga* treated animals compared to untreated groups was observed, indicating hypolipidemic effect. The levels of VLDL and LDL-C were increased in diabetic rats which were decreased significantly in treated groups. The treated groups exhibited an increased level of HDL-C than the diabetic control group concluding that the hydroethanolic extract of *K. galanga* possesses hypolipidemic activity and hence can be studied further for the management of diabetes and diabetes-induced complications.

REFERENCES

1. Kottireddy S, Koora S, Selvaraj J. Lupeol exerts its antidiabetic activity through insulin receptor and glucose transporter-4 in gracilis muscle in high fat and sucrose-induced diabetic rats. Drug Invention Today 2019;11:2258-64.
2. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys Ther 2008;88:1322-35.
3. Unnikrishnan R, Anjana RM, Mohan V. Diabetes mellitus and its complications in India. Nat Rev Endocrinol 2016;12:357-70.
4. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, *et al*. Type 1 diabetes mellitus. Nat Rev Dis Primers 2017;3:17016.
5. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in Type 2 diabetes. Diabetes Care 2009;32 Suppl 2:S157-63.
6. Baskaran K, Pugalendi KV, Saravanan R. Antidiabetic and antihyperlipidemic activity of chrysoeriol in diabetic rats, role of HMG CoA reductase, LCAT and LPL: *In vivo* and *in silico* approaches. J Pharm Res 2015;9:597-605.
7. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med 2011;8:1-0.
8. Nayak A, De S. Antidiabetic potential of medicinal plants. BioMedRx 2013;1:32-46.
9. Kumar KM, Asish GR, Sabu M, Balachandran I. Significance of gingers (*Zingiberaceae*) in Indian system of medicine Ayurveda: An overview. Anc Sci Life 2013;32:253-61.
10. Preetha TS, Hemanthakumar AS, Krishnan PN. A comprehensive review of *Kaempferia galanga* L. (*Zingiberaceae*): A high sought medicinal plant in tropical Asia. J Med Plants Stud 2016;4:270-6.
11. OECD Guidelines for Testing of Chemicals 423. Acute Oral Toxicity Acute Toxic Class Method; 2001.
12. Ramachandran S, Asokkumar K, Maheswari MU, Ravi TK, Sivashanmugam AT, Saravanan S. Investigation of antidiabetic, antihyperlipidemic, and *in vivo* antioxidant properties of *Sphaeranthus indicus* Linn. in Type 1 diabetic rats: An identification of possible biomarkers. Evid Based Complement Alternat Med 2011;2011:571-721.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Estimation of protein. J Biol Chem 1951;193:265-75.
14. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-5.
15. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen

peroxide. Clin Chem 1982;28:2077-80.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
17. Scoppola A, Montecchi FR, Menzinger G, Lala A. Urinary mevalonate excretion rate in Type 2 diabetes: Role of metabolic control. Atherosclerosis 2001;156:357-61.
18. Nithiya T, Udayakumar R. Hepato and renal protective effect of phloretin on streptozotocin induced diabetic rats. J Biomed Pharm Sci 2018;1:105.
19. Bandawane D, Juvekar A, Juvekar M. Antidiabetic and antihyperlipidemic effect of *Alstonia scholaris Linn.* bark in streptozotocin induced diabetic Rats. Ind J Pharm Educ Res 2010;45:114-20.
20. Ramachandran S, Naveen KR, Rajinikanth B, Akbar M, Rajasekaran A. Antidiabetic, antihyperlipidemic and *in vivo* antioxidant potential of aqueous extract of *Anogeissus latifolia* barks in Type 2 diabetic rats. Asian J Pac Trop Dis 2012;2:596-602.
21. Saleem M, Tanvir M, Akhtar MF, Iqbal M, Saleem A. Antidiabetic potential of *Mangifera indica* L. cv. anwar ratol leaves: Medicinal application of food wastes. Medicina (Kaunas) 2019;55:353.
22. Pari L, Amarnath Satheesh M. Antidiabetic effect of *Boerhavia diffusa*: Effect on serum and tissue lipids in experimental diabetes. J Med Food 2004;7:472-6.
23. Eddouks M, Lemhadri A, Michel JB. Hypolipidemic activity of aqueous extract of *Capparis spinosa* L. in normal and diabetic rats. J Ethnopharmacol 2005;98:345-50.
24. Mironova MA, Klein RL, Virella GT, Lopes-Virella MF. Anti-modified LDL antibodies, LDL-containing immune complexes, and susceptibility of LDL to *in vitro* oxidation in patients with Type 2 diabetes. Diabetes 2000;49:1033-41.
25. Markku L. Epidemiology of diabetic dyslipidemia. Diabetes Rev 1995;3:408-22.
26. Jarald EE, Joshi SB, Jain DC. Antidiabetic activity of flower buds of *Michelia champaca* Linn. Indian J Pharmacol 2008;40:256-60.
27. Kameswararao B, Kesavulu MM, Apparao C. Evaluation of antidiabetic effect of *Momordica cymbalaria* fruit in alloxan-diabetic rats. Fitoterapia 2003;74:7-13.
28. Pushparaj PN, Low HK, Manikandan J, Tan BK, Tan CH. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. J Ethnopharmacol 2007;111:430-4.
29. Venkateswaran S, Pari L, Saravanan G. Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in rats with streptozotocin-induced diabetes. J Med Food 2002;5:97-103.

Source of support: Nil; Conflicts of interest: None Declared