



Effect of Katakakhadiradi Kashayam on Lipid Profile and Pancreatic Damage in Type II Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. Author AJSS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MK and MR managed the analyses of the study. Authors KP and MRKR managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i28B31547

Editor(s):

(1) Mohamed Fathy, Assiut University, Egypt.

Reviewers:

(1) Kihong Hong, Seoul Veterans Hospital, Republic of Korea.

(2) Alireza Mohtashami, Islamic Azad University, Iran.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/68461>

Original Research Article

Received 02 March 2021

Accepted 09 May 2021

Published 12 May 2021

ABSTRACT

The lipid pattern and levels in diabetic patient are the same as those for subjects with cardiovascular disease. The tactic underlying the oral hypoglycemic agent is to adjust the lipid profile; which can be achieved by herbal therapy. The use of herbs and formulations for attenuation of hyperglycemia and to aid protection against the pancreatic damage is clinically very important. This study was intended to find the efficacy of *Katakakhadiradi Kashayam*(KKK) on lipid profile and pancreatic tissue damage in streptozotocin-nicotinamide (SN) induced diabetic rats. The diabetic rats were treated with *Katakakhadiradi Kashayam* orally at doses of 100, 200 and 300 mg/kg/bw. For

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28 days and compared with the standard drug Glibenclamide. After the kashayam treatment triglyceride, HDL-cholesterol and total cholesterol level were assayed and pancreatic tissue damage caused by streptozotocin was analysed by histology study. *Katakakhadiradi kashayam* could restore the serum lipid profile by controlling the blood glucose level and reduce the pancreatic injury in diabetic rats. Supplementation of *Katakakhadiradi kashayam* showed a significant improvement in the serum lipid profile thus helping in retarding the secondary complications.

Keywords: Diabetes; hyperlipidemia; *katakakhadiradi kashayam*; pancreas.

1. INTRODUCTION

Diabetes (T2DM) and associated cardiovascular complications are major public health challenges globally. Chronic inflammation of the pancreas in diabetic condition can damage the beta cells that secretes insulin. People with T2DM have two- to four-fold enhanced risk of coronary artery disease (CAD), the leading cause of death in people with T2DM [1]. Hypertension and dyslipidemia are major modifiable risk factors for T2DM and connected CAD, which account for more than 87% of disability in low- and middle-income nations [2].

High lipid levels are related with serious health risks and elevated mortality. Hypertension, insulin resistance, hyperlipidemia and glucose intolerance are recognized as cardiac risk aspects in diabetes patients. The persistence of hypercholesterolemic state causes increased oxidative stress, resulting in the progression of coronary artery disease and other complications of diabetes [3].

The cholesterol together with some other types of fats cannot be dissolved in the blood. Furthermore, in order to be shifted to and from cells, they have to be specially carried by specific molecules known as lipoproteins, which has an outer layer of protein with an inner core of cholesterol and triglycerides. Moreover, lipoproteins are found essential for cholesterol to travel around the body [4].

Lipid alterations in patients with diabetes, often known as “diabetic dyslipidemia”, which are typically characterized by high triglycerides, high total cholesterol, low high density lipoprotein cholesterol and elevated levels of small dense LDL particles [5]. Low density lipoprotein cholesterol levels may be moderately enhanced or normal. Lipid abnormalities are common in people with T2DM and prediabetes but the pattern of the different lipids may alter between economic levels, ethnic groups, and access to health care [6].

Recently, there is growing interest in herbal medicines all over the world. Traditional herbs having antilipidemic effect and antidiabetic effect can prove to be useful source for the development of new oral hypolipidemic and hypoglycemic agents or simple dietary adjuvant to existing therapies [7]. Although *Katakakhadiardi kashayam* has not been studied for its biological activity, but the plant components were reported to contain flavanoids, lignins, alkaloids, triterpenoids and polyphenolic compounds [8]. *Katakakhadiardi kashayam* treatment was found to have beneficial effect on hyperglycemia, blood glucose level restored to a normal clinical range. The present work was designed to study the effect of *Katakakhadiardi kashayam* on lipid profile in streptozotocin induced diabetic rats. To evaluate the effect of *Katakakhadiardi kashayam* on the histological changes which occur in the pancreatic tissue of the diabetic rats and the possible mechanisms through which this formulation produces its antidiabetic effect.

2. METHODS

2.1 Plant Materials and Formulation

All the plant materials were collected from the herbal garden and drug store of Ayurveda College, Coimbatore, Tamil Nadu, India. *Katakakhadiradi Kashayam* is a herbal decoction prepared from each 10 grams of the following ingredient plants. *Strychnos potatorum*, *Acacia catechu*, *Embelica officinalis*, *Berberis aristata*, *Biophytum sensitivum*, *Barringtonia acutangula*, *Cyperus rotundus*, *Salacia reticulata*, *Curcuma longa*, *Terminalia chebula*, *Mangifera indica* and *Cyclea peltata*.

2.2 Animals

Adult male albino wistar rats (6 weeks), weighing 150 to 200 g were used for the present antidiabetic study. The rats were kept in clean polypropylene cages and maintained in a well-ventilated temperature regulated animal cage with a continuous 12 h light/dark schedule. The

rats were given standard rat pelleted diet and filtered drinking water was given *ad libitum*.

2.3 Experimental Induction of Diabetes

The rats were kept overnight fasting and checked the primary fasting blood glucose from the blood sample collected from the tip of rat tail vein. The streptozotocin (60 mg/kg) was dissolved in citrate buffer (pH 4.5) and intraperitoneally injected to the rats done overnight fasting, after 15 min and 120 mg/kg of nicotinamide was administered intraperitoneally to induce non-insulin dependent DM. Hyperglycemia was noted after 72 hrs by the rise in blood glucose levels. The rats with blood glucose level higher than 250 mg/dl were used for the present study [9].

2.4 Study Design

The rats were classified into 6 groups each having six animals. Group I rats received normal saline 1 ml/kg bw. orally; Group II, animals for diabetes induction experimentally injected with inj streptozotocin 60 mg/kg bw. along with nicotinamide 120 mg/kg as intraperitoneal injection; Group III diabetic rats treated with Glibenclamide 20 mg/kg orally for 28 days; Group IV diabetic rats treated with *Katakakhadiradi kashayam* (KKK) 100 mg/kg orally for 28 days; Group V diabetic rats treated with KKK 200 mg/kg orally for 28 days; Group 6 diabetic rats treated with KKK 300 mg/kg orally for 28 days.

2.4.1 Blood collection

About 3 ml of blood was collected in a heparinized syringe. The rest of the heparinized sample was transferred to microcentrifuge tube and run at 5000 rpm for 20 min at 4°C to obtain serum. The serum was transferred to a clean microcentrifuge tube and stored at -80°C (Kumar et al., 2017).

2.4.2 Estimation of total cholesterol

Cholesterol estimation was done conferring to the procedure of Parekh and Jung, (1970).

Ferric chloride-uranyl acetate reagent (2.9 ml) was added to sample (0.1 ml). After centrifugation, sulphuric acid-ferrous sulphate reagent (2.0 ml) was added gradually and mixed well. Blank containing ferric chloride-uranyl acetate reagent (3.0 ml) and sulphuric acid - ferrous sulphate reagent (2.0 ml). A calibration

graph was made with standard cholesterol. In a Shimadzu UV spectrophotometer, the optical density was taken (530 nm after 20 minutes). The levels of cholesterol were measured in mg/dl [10].

2.4.3 Estimation of triglycerides

Triacylglycerol estimation was done according to the procedure of Rice, (1970). To 0.1 ml sample, activated alumina (50 mg) and isopropanol (3.9 ml) was added and mixed well. This was then kept for 15 minutes. Centrifugation was done and supernatant (2.0 ml) was taken for examination. Alkaline potassium hydroxide(0.6 ml) was added to every tube and were kept for 10 minutes at 60°C. The tubes were chilled and sodium metaperiodate (1.0 ml) reagent was added to the tubes followed by the adding acetyl acetone reagent (0.5 ml). The tubes were again cooled and the color formed was read in a Shimadzu UV spectrophotometer at 405 nm against blank. The levels of triacylglycerol were measured in mg/dl [11].

2.4.4 Estimation of HDL

The HDL-consisting supernatants and the wholeserum test samples were assayed in opposed to the otclipoprotein antiserum as 20- and 40-fold dilutions, respectively, in aqueous sodium chloride (150 mmol/l). In assays against the lipoprotein antiserum, sera were diluted 20-fold with the same solution but the supernatants were applied undiluted. All precipitated applicable material was made free from any occluded supernatant before application. Each precipitate was dissolved in a volume of aqueous sodium chloride (150 mmol/l) equal to that of the original serum aliquot and reprecipitated by addition of an equal volume of aqueous PEG solution, as described above. The secondary supernatant was thrown out and the process was redone once more. Lastly, the low-density lipoproteins were re-dissolved in the same volume of aqueous sodium chloride [12].

2.4.5 Masson trichrome Staining

For analysing histopathology of pancreas, the tissues were administered for paraffin sectioning. The sections were fixed in Bouin's solution at room temperature overnight to intensify the colors and increase the contrast between the tissue components. The slides were washed for 1-2 minutes under running tap water (18–26°C) to remove yellow color from sections and then

rinse briefly in double distilled water. The sections were incubated for 5 minutes with Weigert's Iron Hematoxylin solution to stain the nuclei dark and then discarded the solution. The slides were placed in a glass chamber and washed under warm running tap water for 10 minutes to remove excess of Hematoxylin and to intensify the black color. Then incubated the sections for 5 minutes with Biebrich Scarlet-Acid Fuchsin Solution to stain the fibers red. The images are viewed using a bright-light microscope (40x magnification) and photomicrographs were taken using a Sony digital camera.

2.5 Statistical Analysis

The mean value and standard error were measured for each parameter. The significant differences were observed, mean values were compared using one way ANOVA. A probability value of less than 0.05 was considered significant.

3. RESULTS

Figs. 1-3 depicts that there was a significant ($p < 0.001$) increase in triglyceride, total cholesterol and ($p < 0.001$) significant decrease in high density lipoprotein in diabetic rats. The present study showed that Glibenclamide ($p < 0.001$) significant reduction in the serum lipid levels in diabetic rats. Similarly, katakakhadiradi

kashayam intake from minimum to maximum doses (100, 200 and 300 mg/kg) significantly restored the serum lipid levels in near normalcy in diabetic rats (Figs. 1-3).

Histopathology results showed that normal control group has normal pancreatic acini with thin sheet of collagen around them. Diabetic rats showed shrunken islets with thick and dense collagen fiber layers seen around the pancreatic ductules (arrow mark) and acini indicative of fibrosis. Glibenclamide group showed normal islets and thin collagen fibres around the acinar cells and ductules. KKK at doses 100 and 200 mg of showed normal islets with mild thickening of collagen fibers around the ductules and in between the pancreatic acini. High dose 300mg KKK showed normal islets with thin layer of collagen fibers around the ductules (Fig. 4).

4. DISCUSSION

Dyslipidemia (or diabetic dyslipidemia when diagnosed in T2DM patients) is an abnormality in lipid metabolism; i.e. quantitatively observed in abnormalities in blood lipids including increased triglycerides (TG) and low-density lipoprotein (LDL-C) and a decreased high-density lipoprotein cholesterol (HDL-C) concentration. These disturbances are established risk factors in the development of atherosclerosis. Dysregulation of intestinal lipoprotein metabolism is often seen in T2DM patients.

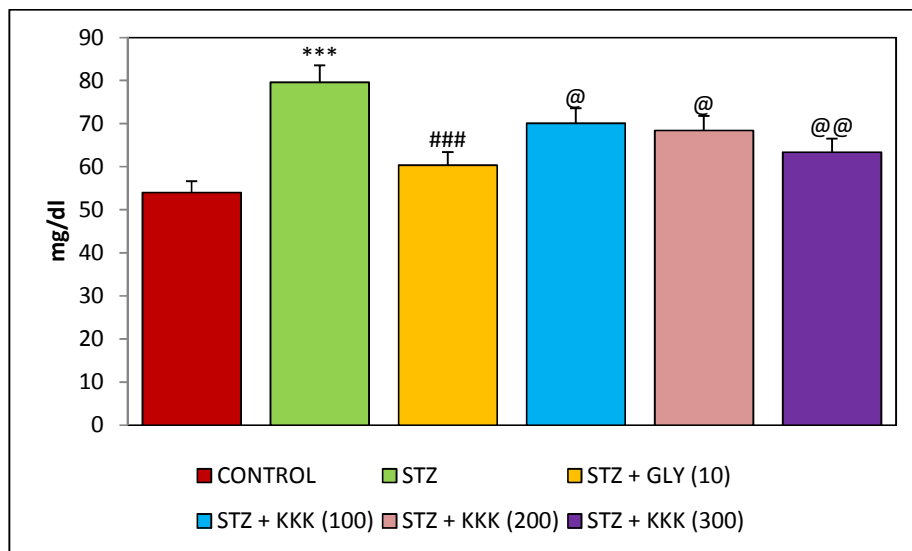


Fig. 1. Effect of katakakhadiradi kashayam on total cholesterol level in diabetic rats
 Values are expressed as mean \pm SEM (n=6); *** $p < 0.001$ significant as compared with control group;
 ### $p < 0.001$, @ $p < 0.05$, @@ $p < 0.01$ significant as compared with diabetic group

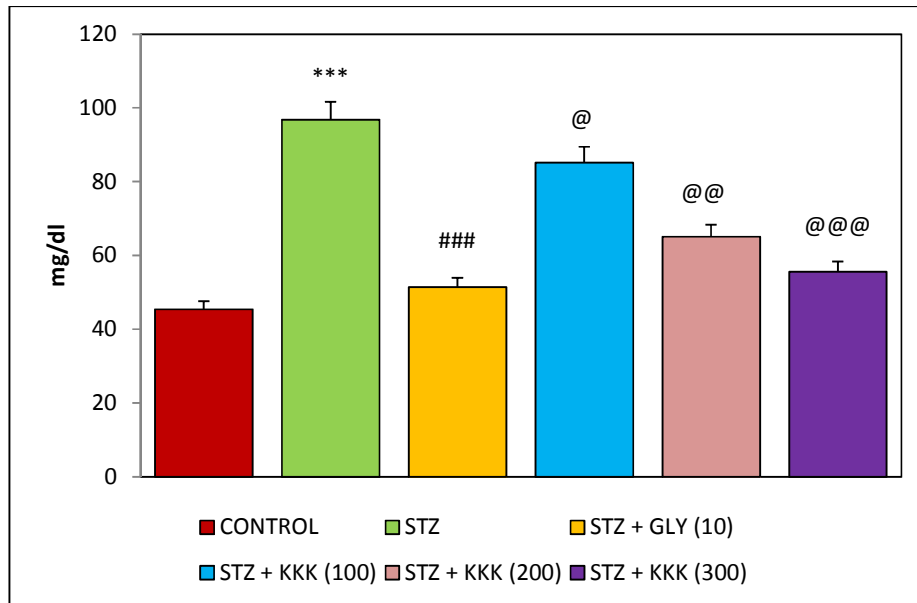


Fig. 2. Effect of katakakhadiradi kashayam on triglyceride level in diabetic rats
 Values are expressed as mean \pm SEM (n=6); ***p<0.001 significant as compared with control group; ###p<0.05, @p<0.05, @@p<0.01, @@@p<0.001 significant as compared with diabetic group

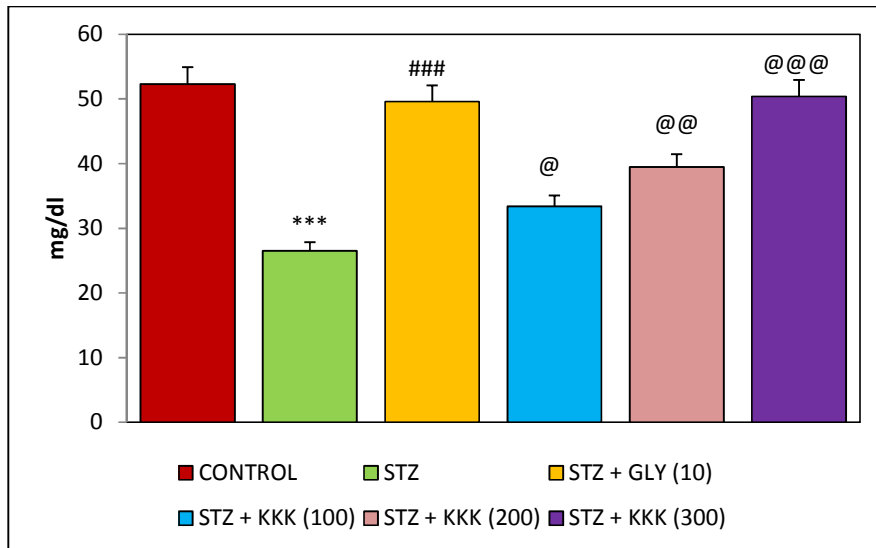


Fig. 3. Effect of katakakhadiradi kashayam on HDL level in diabetic rats
 Values are expressed as mean \pm SEM (n=6); ***p<0.001 significant as compared with control group; ###p<0.05, @p<0.05, @@p<0.01, @@@p<0.001 significant as compared with diabetic group

The main components of katakakhadiradi kashayam are *Acacia catechu*, *Strychnos potatorum*, *Berberis aristata*, *Cassia mimosoides* and *Embelica officinalis* already proved medicinal plants for diabetes and secondary complications related to altered lipid levels [13,14]. The antidiabetic components and antilipidemic components of this formulation are previously

tested by GC-MS analysis and its invitro free radical scavenging activity were also proved [15,16].

Acacia catechu known to be effective in decreasing plasma cholesterol and can inhibit LDL oxidation. *Embelica Officinalis*, *Terminalia chebula*, *Curcuma longa* and *Mangifera indicain*

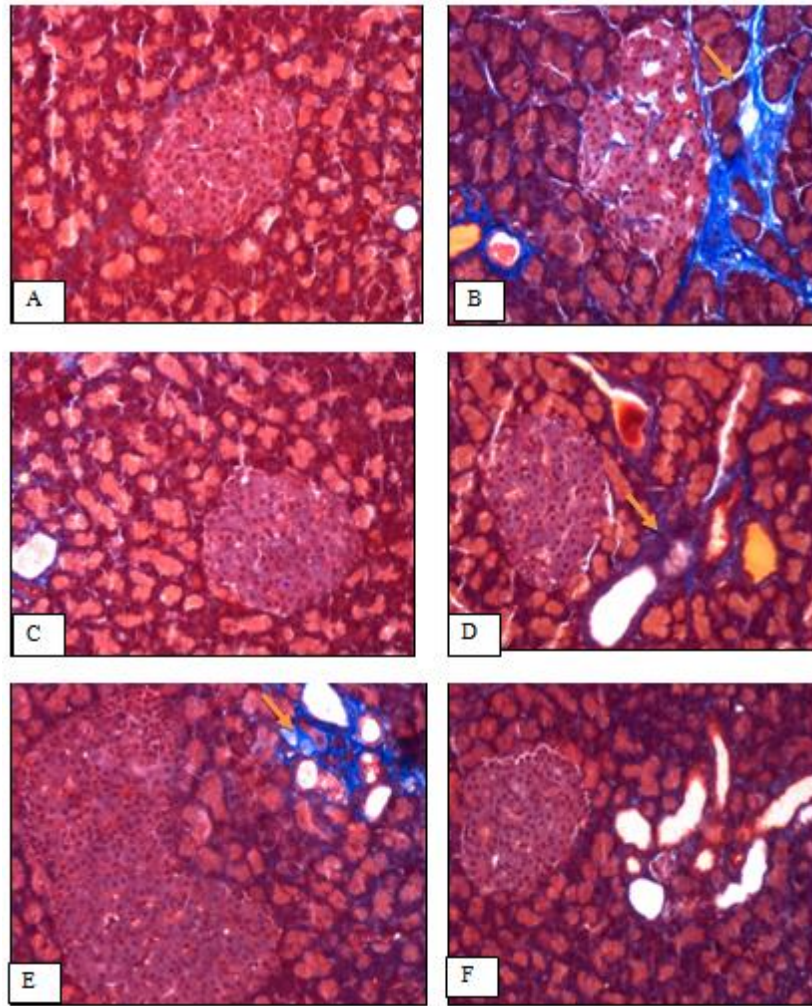


Fig. 4. Histology of pancreas tissue stained by masson trichrome: A: Control (40x), B: STZ+NTA (40x), C: STZ+NTA+GLY (10) (40x), D: STZ+ NTA+KKK (100mg) (40x), E: STZ+NTA+ KKK (200 mg) (40x), F: STZ+ NTA+KKK (300 mg) (40x)

this polyherbal formulation have more antioxidant constituents which prevents the pancreatic cell injury in diabetes condition. The findings are consistent with earlier report showed that administration of Glibenclamide at a dose of 0.6 mg/Kg bw to diabetic rats caused a significant decrease in total lipids [12].

The KKK reduces the lipids and lipoprotein levels in streptozotocin and nicotinamide induced diabetic rats and it is also confirmed in pancreatic tissues by Masson trichrome staining. The KKK at a dose of 300 mg reduces the blood glucose and insulin levels which is already published by the author. The ayurvedic herbs present in the formulation also plays a very important role to

reduce the blood lipids and regulate the lipid metabolism in a well balanced manner.

5. CONCLUSION

This Katakakhadiradikashayam showed perceived effectiveness with fewer side effects and relatively low costs herbal drugs prescribed for diabetes and its associated disease. Therefore, Katakakhadiradi kashayam can be used as current hypoglycemic formulation to improve management of diabetic patients associated with hyperlipidemia.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animal experiments were conducted after getting approval from the ethical committee and in accordance with the guidelines for the appropriate care and use of laboratory animals (IAEC No: KMCRET/Ph.D/16/2016-2017).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:
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