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Effect of Katakakhadiradi kashayam on liver metabolism in streptozotocin and nicotinamide induced diabetic rats

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ABSTRACT

Plants have the ability to impart therapeutic action on chronic diseases such as diabetes and its comorbidities. The current study was done to find the effectiveness of Katakakhadiradi kashayam on liver metabolism in streptozotocin and nicotinamide administered diabetic rats. Diabetic rats were treated with katakakhadiradi kashayam for 28 days and compared with glibenclamide. The antidiabetic action of Katakakhadiradi kashayam was measured by the serum levels of urea, bilirubin, SGOT and SGPT activity. In the current study, the level of urea, SGPT and SGOT activity was significantly higher and reduced total bilirubin observed in diabetic rats. The protection given by Katakakhadiradi kashayam against diabetic complication was confirmed by a histology study. Most of the plants present in Kadakakhadiradi kashayam are effective in regulating the enzymes in diabetes mellitus. The obtained data imply the antidiabetic effects of katakakhadiradi kashayam, which is practically a safe herbal formulation and can be used as a good alternative for managing DM.

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INTRODUCTION

The endocrine, metabolic disorder diabetes, is characterized by hyperglycemia, change in lipids, carbohydrates, protein metabolism and it elevates the risk of cardiopathy complications. The common liver enzymes alteration characterized by additional fat deposition in the liver and related to diabetes mellitus (DM) (Castro *et al.*, 2017). The specific marker enzymes for liver injury are accountable for glutathione catabolism and related to chronic inflammation and oxidative stress.

Recently, DM is measured by various drugs, including insulin and oral hypoglycemic agents, but they had their limitations. Conventionally, various herbal drugs and medicinal plants are used for treating DM

as an alternative medicine. The existence of various phytoconstituents in medicinal herbs is believed to act on various series of targets by several modes and mechanisms. Hence, herbs have the ability to impart therapeutic action in complicated disorders like DM and its comorbidities (Nazarian-Samani *et al.*, 2018).

Many medicinal herbs, conventionally used for over 100 decades named Rasayana (formulation), are present in herbal preparations of Indian traditional health care systems. Katakakhadiradi Kashayam is used for curing DM and urinary ailments (Ighodaro *et al.*, 2017). It is used for relieving diabetes comorbidities, including neuropathy and is believed to regulate both Vata and Kapha related diseases. Many of the components of Katakakhadiradi Kashayam have been reported to have antioxidant effects (George and Nazeema, 2018). Therefore, the standardization process for evaluation of safety, therapeutic risk and advantage is related to utilization of a polyherbal formulation is an area that requires considerable attention. The present study was done to find the effectiveness of Katakakhadiradi kashayam on liver function alteration associated with diabetes.

Methodology

Plant materials and formulation

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

Animals

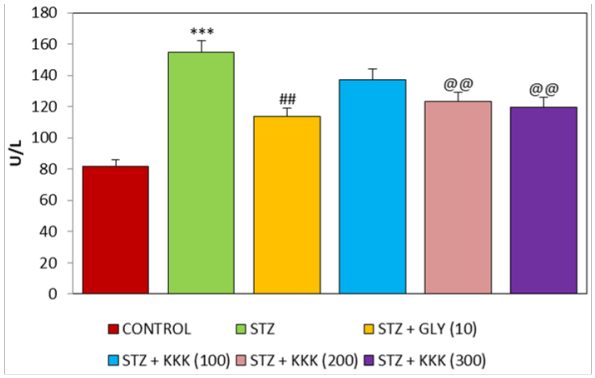
Adult male albino Wistar rats (6 weeks) of 150 to 200 g were taken for the present antidiabetic study. The rats were kept in clean polypropylene cages and provided with a well-ventilated temperature regulated animal cage with a continuous 12 h day/night schedule. The rats were given a standard rat pelleted diet and filtered water was given *ad libitum*. Every animal experiments were conducted after getting permission from the ethical committee following the guidelines for the appropriate care and utilization of laboratory animals (IAEC No: KMCRET/Ph.D/16/2016-2017).

Experimental induction of diabetes

The rats were hold fasting overnight and checked the primary fasting blood glucose from the rat's tail vein at the tip. The streptozotocin dissolved in citrate buffer (pH 4.5) and nicotinamide dissolved in normal saline was used to induce diabetes. The streptozotocin (60 mg/kg) was suspended in citrate buffer (pH 4.5) and intraperitoneally injected into the rats done overnight fasting, after 15 min and 120 mg/kg, nicotinamide was administered intraperitoneally to induce non-insulin dependent DM. Hyperglycemia was observed after 72 hours by the increased blood glucose levels. The rats with blood glucose level higher than 250 mg/dl were taken for the present study.

Study Design

The rats were classified into 6 groups, each having six animals. Group, I rats was administered normal saline 1 ml/kgb.wt orally; Group II rats injected with streptozotocin 60 mg/kg/b.w and nicotinamide 120 mg/kg intraperitoneally to induce diabetes; 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 Katakakhadiradi kashayam (KKK) 300 mg/kg orally for 28 days.



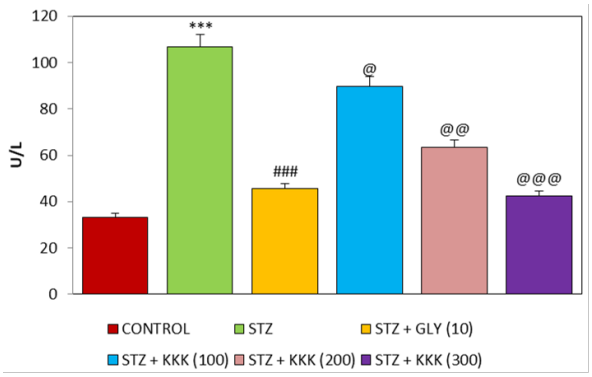
Values are measured as mean ± SEM Statistical significance (n=6); ***p<0.01 significant in comparison with control group; ###p<0.05, @p<0.05, @@p<0.01 @@@p<0.001 significant in comparison with diabetic group.

Figure 1: Effect of katakakhadiradi kashayam on SGOT activity in streptozotocin induced diabetic rats

Blood collection

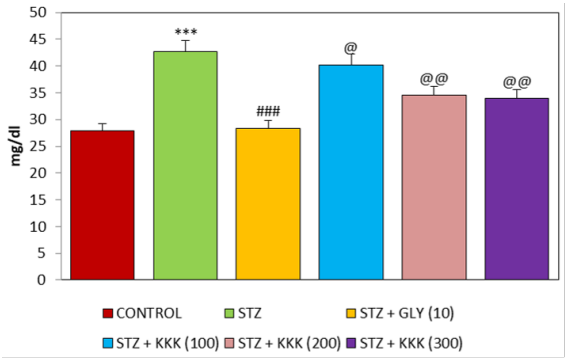
About 3 ml of blood was taken in a heparinized syringe. The rest of the heparinized sample was transferred to a microcentrifuge tube and run for 20 min at 5000 rpm (4° C) to obtain serum. The serum was shifted to a clean microcentrifuge tube and incubated at −80° C (Kumar *et al.*, 2017).

Assay of Serum Glutamate Oxaloacetate Transferase



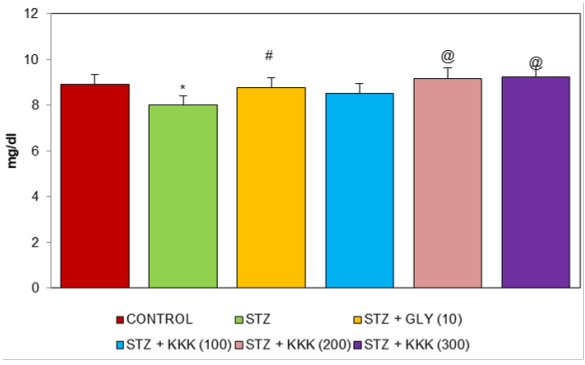
Values are measured as mean ± SEM (n=6); ***p<0.01 significant in comparison with control group; ###p<0.001, @p<0.05, @@p<0.01, @@@p<0.001 significant in comparison with diabetic group.

Figure 2: Effect of katakakhadiradi kashayam on SGPT activity in streptozotocin and nicotinamide induced diabetic rats



Values are measured as mean ± SEM (n=6); ***p<0.001 significant in comparison with control group; ###p<0.001, @p<0.05, @@p<0.01, significant when compared to diabetic group.

Figure 3: Effect of katakakhadiradi kashayam on urea level in streptozotocin and nicotinamide induced diabetic rats



Values are measured as mean ± SEM (n=6); *p<0.05 significant in comparison with control group; #p<0.05, @p<0.05 significant in comparison with diabetic group.

Figure 4: Effect of katakakhadiradi kashayam on total bilirubin in streptozotocin and icotinamide induced diabetic rats

To 0.1 ml of serum, a buffered substrate (1 ml) was added and kept for 1 hour at 37ºC. To arrest the reaction, the DNPH reagent (1 ml) was added and the tubes were incubated for 15 minutes, and then 10.0 ml of sodium hydroxide was added and measured at 520 nm in a Shimadzu UV spectrophotometer. The enzyme activity was measured in IU/L serum (Perez *et al.*, 2020).

Assay of Serum Glutamate Pyruvate Transferase

The methods and reagents used were similar to those used for aspartate transaminase analysis, excluding the substrate solution and incubation time being decreased to 30 minutes. The substrate mixture includes2-oxoglutarate (38 mg) and DL-alanine (1.78 g) were softened in the buffer, sodium hydroxide (0.5 ml) was added and the volume was prepared up to 100 ml with buffer and the same procedure was repeated like AST. The enzyme activity was measured in IU/L serum (Perez *et al.*, 2020).

Estimation of bilirubin

To 200 μl of serum, diazo reagent (1.8ml) was added, to this mixture, methanol (2.5ml) was added and allowed to stand at room temperature. The bilirubin stock solution was diluted with methanol to give a final concentration of 2mg/100ml. Blank serum was substituted by HCl (1.5%). The values were measured in mg/dl of serum (Putluru *et al.*, 2016).

Estimation of urea

By using an enzymatic colorimetric test of Berthelot, blood urea was determined. Urea gets hydrolysed in the presence of water and urease and generates ammonia and carbon dioxide. The blank reaction was performed initially: 0.02 ml of distilled water was pipetted into the cuvette and working reagent (2 ml). To the cuvette, reagent (2ml) and serum sample (0.02 ml) was pipetted out, absorbance was taken and the concentration of urea was calculated. In an altered Berthelot reaction, a green dye has been made through the reaction of ammonium ions when reacted with hypochlorite and salicylate. At 578 nm, the absorbance increases and is proportional to urea concentration in the sample.

Hematoxylin and Eosin Staining

For analysing histopathology, liver tissues were administered for paraffin sectioning. Then the tissues were hydrated and dehydrated in sorted alcohol series. It was then cleared using xylene and chloroform, and then it was fixed in paraffin wax using a rotary microtome, tissues sections were taken (10 μm) out and kept overnight at room temperature. It was then deparaffinised and moistened with descending alcohol concentrations fol-

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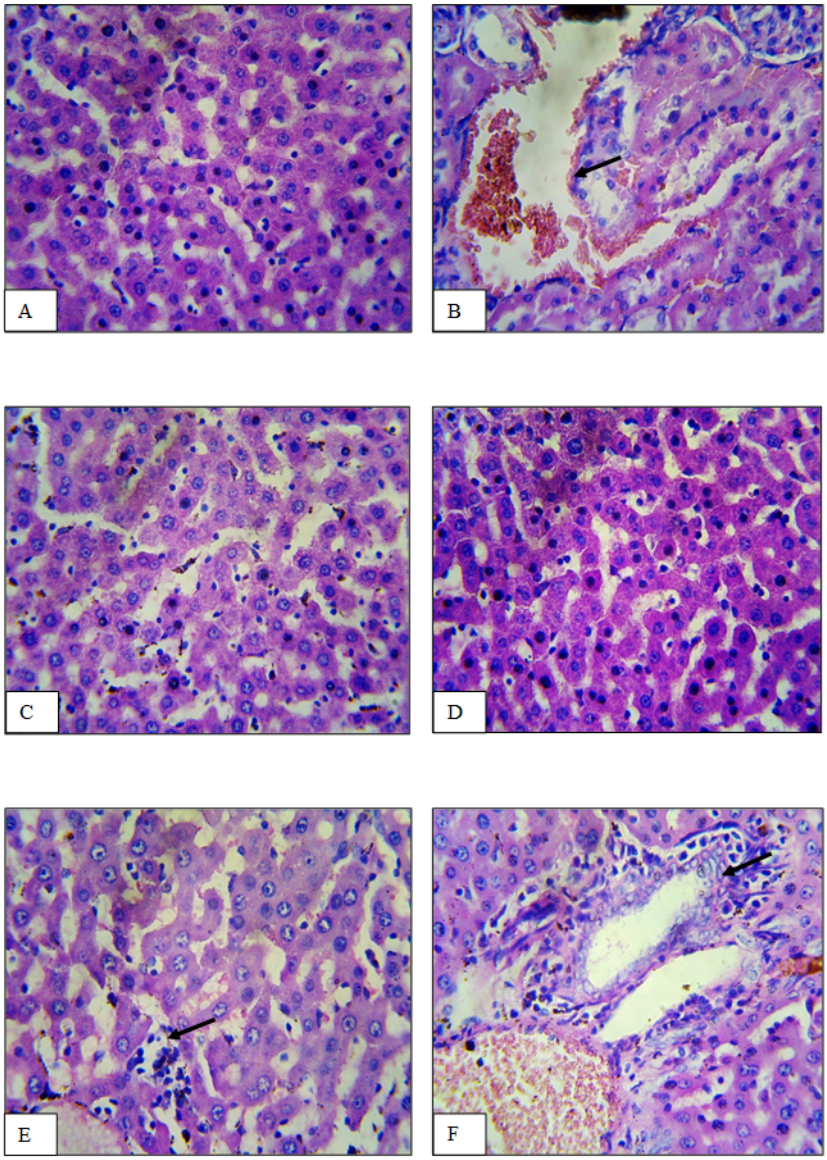

A: Control (40x), B: STZ (40x), C: STZ+GLY (10 mg) (40x), D: STZ+ KKK (300 mg) (40x), E: STZ+ KKK (200 mg) (40x), F: STZ+ KKK (100mg) (40x).

Figure 5: Histology of liver tissue stained by Hematoxylin and Eosin

lowed by dist.H_2O. Using haematoxylin and eosin stain, the sections were stained and then administered for ascending alcohol concentrations. The permanent slide was prepared using a DPX mount. The slides were observed under a light microscope (20x) (Olympus microscope) and photomicrographs were taken using a Sony digital camera.

Statistical analysis

The mean value and standard error were measured for each parameter. The analysis was carried out by using SPSS software (version 21.0). The significant differences were observed, mean values were compared using one way ANOVA. A P value of below 0.05 is considered statistically significant.

RESULTS AND DISCUSSION

The liver organ has the main role in carbohydrate metabolism since it is responsible for the stability of blood glucose levels by means of glucose metabolic cycles. The enhanced effects of liver enzymes like alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT) and aspartate aminotransferase (AST) are signals of hepatocellular damage. Enhanced effect of these markers is related to metabolic syndrome, insulin resistance, and diabetes type 2 (Madhavan *et al.*, 2019). Bilirubin has a diverse role in antioxidant, anti-inflammatory, and immunological properties and defends against cardiovascular and microvascular complications

related to diabetes (Inoguchi *et al.*, 2019). Evolving epidemiologic evidence proposes that higher urea levels are connected with an elevated risk of diabetes mellitus incidence (Xie *et al.*, 2018). The trend between serum urea levels and blood sugar in people with diabetes has shown a strong association, as evidenced in a study by (Bamanikar *et al.*, 2016). The abnormal renal function, as shown by a reduction in glomerular filtration and a rise in serum urea and creatinine levels (Aguiar *et al.*, 2016). In diabetic nephropathy, biomarkers viz. serum urea seems to be increased with hyperglycemia in uncontrolled diabetics and generally associate with kidney damage severity (Mohapatra and Damodar, 2016). Because of poor blood glucose control, fat gets deposited in the liver, which may result in the elevation of SGOT and SGPT enzymes. Controlling blood glucose may improve liver protection by normalising the marker enzyme levels in serum (Jayant and Srivastava, 2016).

In the present study, the effect of SGOT ($p<0.05$), SGPT ($p<0.001$), and urea ($p<0.01$) levels were observed to be significantly higher and reduced levels of total bilirubin ($p<0.05$) were found in the diabetic rats in comparison with normal control. Katakakhadiaradi kashayam treated rats with a dose of 300 mg/kg showed better results similar to standard compared to the diabetic group (Figures 1, 2, 3 and 4). The KKK dose at 100 mg/kg and 200 mg/kg showed $p<0.05$ and $p<0.01$ significant efficacy against streptozotocin and nicotinamide induced alteration in liver damage. The protection percentage of KKK (300mg) was high in comparison with KKK (100 mg) and (200mg) dose.

The histological study of the liver proved that vehicle treated rats showed normal central vein and hepatic cell morphology. Diabetic rats have shown the disaggregation of trabeculae and extensive vacuolization with nuclei disappearance (arrow mark). Glibenclamide and Katakakhadiradi kashayam (300 mg/kg) treated rats reverted the hepatic damage to normal condition, whereas 200mg dosage showed mild pathological features such as neutrophil infiltration (long arrow) in liver tissue (Figure 5). KKK (100mg) treatment showed vascular congestion, central vein damage and hepatic cell inflammation. The present hepatoprotective activity of this kashayam is probably due to flavonoids, phenolic compounds, polyphenols and more bioactive compounds in Katakakhadiradi kashayam.

This kashayam can decrease lipid and serum glucose levels as well as enhance glucose tolerance and insulin in diabetes. Intake of this kashayam also decreases oxidative stress in liver tissue, signifying that these formulations hinder pathological damage and may diminish the late complications of diabetes (Shengule *et al.*, 2018).

CONCLUSIONS

The antidiabetic effect of the polyherbal formulation, Katakakhadiradi kashayam, was proved in this study by minimising the diabetes associate liver damage. Therefore, physicians can rely on Katakakhadiradi kashayam as complementary therapeutics, together with current hypoglycemic drugs for improving the management of diabetic patients.

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The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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