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# Hypoglycemic effect of katakakhadiradi kashayam by alleviating the oxidative damage in experimentally induced diabetic rats

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ABSTRACT

At present, herbal plants and their biologically active components have acquired importance in diabetes mellitus (DM) management, which has spread worldwide**.** The current study was carried out to analyse the antidiabetic action of Katakakhadiradi kashayam in streptozotocin administered diabetic rats. The study was done in wistar rats by inducing diabetes using streptozotocin and nicotinamide. Diabetic rats were given katakakhadiradi kashayam with various doses of 100, 200 and 300 mg/kg/b.wt for 14 days, and its efficacy was compared with glibenclamide drug. The hypoglycemic effect of this katakakhadiradi kashayam was tested by taking blood glucose measurement in experimental rats. The oxidative damage caused by streptozotocin was analysed by LPO levels and the antioxidants status was assessed by GSH levels, GPx, SOD and CAT activities in pancreatic tissues. The antidiabetic study of katakakhadiradi kashayam showed reduction in hyperglycemia by reducing the oxidative damage in pancreatic tissue and improving the antioxidants. Overall, the reports of the study showed that katakakhadiradi kashayam could be used to improve management of diabetic rats. The acquired data suggest the hypoglycemic efficacy of katakakhadiradi kashayam, which is practically a safe herbal formulation and may be used as a good alternative to cure diabetes mellitus.

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## INTRODUCTION

Diabetes mellitus (DM) is a clinical disorder leads to hyperglycemia, higher glycated haemoglobin, with a higher risk of mortality and morbidity. Diabetes mellitus is a result of diminished efficacy of endogenous insulin or the inappropriate use of insulin by target cells, and is categorized by hypertension, unbalanced metabolism and vasculature (Oguntibeju, 2019).Streptozotocin is a antineoplastic agent that is toxic to the beta cells of the pancreas which generates insulin in mammals. For islets of Langerhans cancer treatment strepto-

zotocin was used and in medical research field for experimental induction of hyperglycemia this drug was used specifically (Alotaibi *et al.*, 2019).

The diabetes treatment has multifactorial intervention including continuous physical action to control cardiac disease, glycemic range, hypertension, which can be achieved by oral hypoglycemic placebo and therapeutic compounds to retard glucose absorption by reducing the secretion of carbohydrate hydrolyzing enzymes like glucosidases and amylase (Tomar *et al.*, 2019; LeRoith *et al.*, 2019).

In the past few decades there has been an exponential development in the area of traditional medicine and these drugs are gaining attention both in developed and developing nations due to their natural origin and lower or no adverse effects. Many traditional medicines in the market are procured from minerals, organic matter and herbal plants. Many herbs, traditionally used for disease cure over hundred decades named rasayana (formulation) are present in herbal preparations of Indian health care systems (Zheng *et al.*, 2019).

One such formulation is katakakhadiradi, which brings about the effect of decreasing blood glucose levels by controlling the pancreas secretions. This kashayam consist of twelve herbs including *Strychnos potatorum, Acacia catechu, Embelica officinalis, Berberis aristata, Biophytum sensitivum, Barringtonia acutangula, Cyperus rotundus, Salacia reticulata, Curcuma longa, Terminalia chebula, Mangifera indica* which possesses many medicinal properties and used for many ailments traditionally.

Many of the components of Katakakhadiradi Kashayam have been reported to possess antioxidant effects (Jessica *et al.*, 2016). This study was carried out to highlight the antidiabetic action of Katakakhadiradi kashayam on streptozotocin and nicotinamide induced diabetic rats and its efficacy was compared with the standard drug glibenclamide.

## 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. Katakakhadiradi Kashayam is an herbal decoction prepared from 10 grams each 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 and Mangifera indica.*

### Animals

Male albino wistar rats (45 days), weighed 150 to 200 g were used for the current antidiabetic study. The rats were placed in sterilized polypropylene cages and the temperature was maintained with a continuous 12 h light cycle and 12h dark cycle. The rats were provided with standard rat diet and RO water *ad libitum*. All animal experiments were conducted after getting approval from the ethical committee and following the guidelines for the appropriate care, experimental induction and use of laboratory rats (IAEC No: KMCRET/Ph.D/16/2016-2017).

### Diabetes induction

The rats were kept overnight fasting and checked the primary fasting blood glucose from the tip of rat vein in the tail region. The streptozotocin and nicotinamide (60 mg/kg) was suspended in citrate buffer adjusted to pH 4.5 and injected intraperitoneally to induce Type II Diabetes mellitus. Hyperglycemia was observed after 72 hours by the increased glucose levels in rats administered with streptozotocin. After 72hrs the rats which showed glucose levels higher than 250 mg/dl were used for the current antidiabetic study (Mahmoud *et al.*, 2017).

### Study Design

The rats were classified into 6 categories each having six animals. Normal control rats were given 1 ml/kg b.wt saline orally; Diabetic rats injected with streptozotocin and nicotinamide 60 mg/kg/b.w. intraperitoneally; The diabetic rats were treated with standard drug glibenclamide 20 mg/kg orally for 28 days; Katakakhadiradi kashayam (KKK) (100, 200 and 300 mg/kg) orally as a single dose for 28 days to diabetic rats.

All through the study, Glibenclamide and Katakakhadiradi kashayam was freshly prepared in normal saline and distilled water before to the treatment. The fasting blood glucose level was assessed on $1^{st}$, $3^{rd}$, $11^{th}$, $15^{th}$ and $28^{th}$ day from vein at the tip of rat tail. Serum glucose levels were measured by spectrophotometry using glucose oxidase method. The serum was subjected to analysis of glucose by commercial kits with the manufacturer's suggestions (Copp *et al.*, 2018).

The animal was sacrificed by cervical dislocation and the pancreas was removed and washed immediately with ice cold saline. About 100 mg of tissue was weighed and homogenized in 5mL of tris-hydrochloride buffer (pH7.4) in ice cold condition. The homogenate was then centrifuged at 2500 xg for 10min at 4ºC using Remi Centrifuge (16x model). The supernatant obtained was then used for the various biochemical assays.

### Estimation of Lipid peroxidation

To measure the oxidative damage, the levels of LPO were quantified with thiobarbituric acid reaction method. For measuring, working solution comprised of thiobarbituric acid, trichloroacetic acid and 0.25 N hydrochloric acid was prepared. To 250 $\mu$L tissue homogenate, 500 $\mu$L working solution was added and kept in boiling water for 10 min, and centrifuged at 3000 rpm. Lastly, 200 $\mu$L of each supernatant was transferred to test tubes and the optical density of samples was assessed at 535 nm. The MDA values were measured in nmol/gram protein.

### Assay of superoxide dismutase

A mixture of 2.5 ml of Tris-HCl buffer, 0.1 ml of EDTA and 0.5 ml of DTPA was prepared. To this mixture, 0.5 ml of pyrogallol was added and the increase in absorbance was read at 420 nm against the blank for 3 min to determine the rate of auto oxidation of pyrogallol. About 100 $\mu$l of tissue homogenate was taken in a separate tube and 2.5 ml of Tris-HCl buffer, 0.1 ml of EDTA and 0.5 ml of DTPA was added. To this mixture, 0.5 ml of pyrogallol was added and the increase in absorbance was read at 420 nm using a spectrophotometer against the blank for a period of 3 min. The reagent blank contained a mixture of 3.1 ml of Tris- HCl buffer, 0.1 ml of EDTA and 0.5 ml of DTPA and this was used to set 100% absorbance. The activity of SOD in tissues is expressed as Units/min/mg of tissue protein. The SOD activity measured following the procedure of Zhang *et al.* (2016).

### Assay of catalase

The catalase activity in pancreatic tissue was measured following the procedure of Hadwan (2018). About 10 $\mu$L of tissue homogenate was taken in a test tube and added 100 $\mu$mol/mL of $H_2O_2$ in 0.05 mmol/L Tris-HCl buffer adjusting to pH 7 and incubated for 10 mins. The reaction was ended by adding 4% ammonium molybdate and the absorbance measured at 410 nm. One unit of catalase activity was expressed as the quantity of enzyme needed to decompose 1 $\mu$mol $H_2O_2$ per min (Hadwan, 2018).

### Assay of glutathione peroxidase

The GPx enzyme activity in pancreatic tissues was assayed following the procedure of Li *et al.* (2018). GPx enzyme was measured depending on the fact that GPx catalyses glutathione oxidation by cumene hydroperoxide. The oxidized glutathione transformed to reduced glutathione due to NADPH oxidation to $NADP^+$. About ten microlitre of tissue homogenate was mixed with 500 microlitre GPx analysing reagent and 20 $\mu$L cumene. The absorbance was read at 340 nm. The enzyme action was measured in U/mg protein.

### Estimation of reduced glutathione

To the test tubes 1.5 mL of precipitating reagent, 100 $\mu$l of sample and 900 $\mu$l of distilled water were added, mixed and incubated for 5 min at 37°C. The test mixture after incubation was centrifuged (4000 rpm$\times$ 4°C $\times$15 min), phosphate solution (4.0 mL) and DTNB (0.5 mL) was added to 1.0 mL of supernatant. The colour changes to yellow colour complex which was read using spectrophotometer (412 nm). The GSH concentration present in the tissue homogenate was measured as $\mu$mol of U/mg protein (Xu *et al.*, 2017).

### Statistical analysis

The results were presented as Mean $\pm$ SEM for six animals in each group for each parameter. The statistical comparison was done by using SPSS software. The significant variation was observed, mean values were related using one-way ANOVA. A p-value less than 0.05 were meant statistically significant.

## RESULTS AND DISCUSSION

Diabetes results in high blood sugar levels via two possible mechanisms: inadequate insulin production in the pancreas, or confrontation to the effect of insulin elsewhere in the body (Bruen *et al.*, 2017). Streptozotocin and nicotinamide induced diabetic rats showed ($p<0.001$) significant hike in glucose levels in comparison with control rats. Glibenclamide treated group showed ($p<0.001$) significant reduction in blood glucose levels similar to normal clinical range. The present study showed that Katakakhadiradi kashayam at 200 and 300 mg/kg treated rats are more effective in balancing the levels of glucose when compared with diabetic rats than its lower dose 100 mg/kg. Figure 1 Data are expressed as mean $\pm$ SEM (n=6); $^{***}p<0.001$ vs control group. $^{\#\#\#}p<0.001$, $^{@}p<0.05$, $^{@@}p<0.01$, $^{@@@}p<0.001$ vs diabetic group.

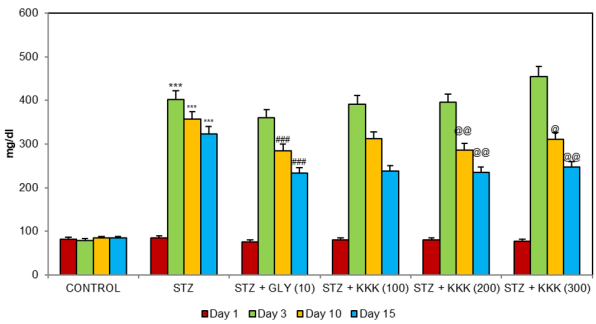


**Figure 1: Katakakhadiradi kashayam efficacy on blood sugar levels in diabetic rats**

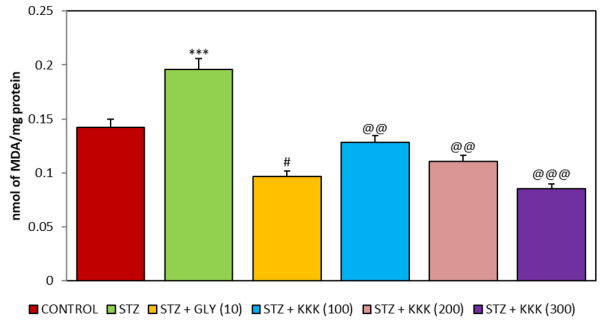


**Figure 2: Effect of katakakhadiradi kashayam on LPO levels in streptozotocin and nicotinamide induced diabetic rats**

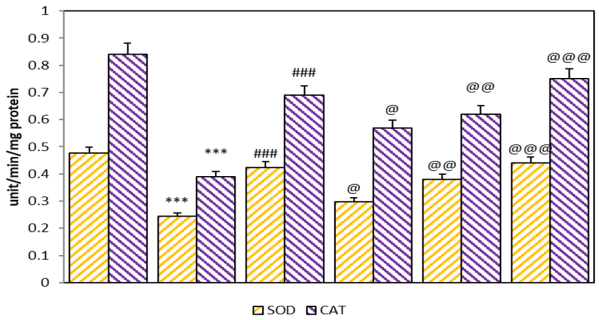


**Figure 3: Effect of katakakhadiradi kashayam on Superoxide dismutase and catalase enzyme activity in streptozotocin and nicotinamide induced diabetic rats**

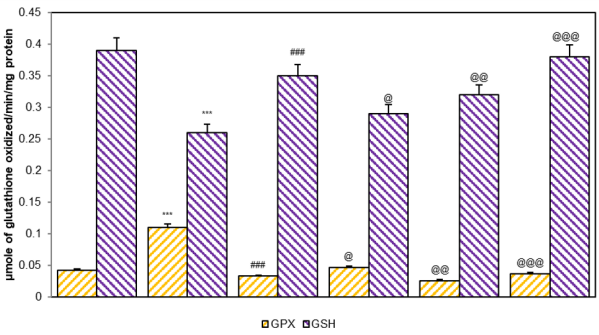


**Figure 4: Effect of katakakhadiradi kashayam on the Glutathione peroxidase activity and Glutathione levels in streptozotocin and nicotinamide induced diabetic rats**

Excessive free radical generation upon streptozotocin induction reduces the action of antioxidant enzymes. The production of free radicals more than the scavenging properties of invivo antioxidant defense enzymes leads to macro- and microvascular dysfunction (Panahi *et al.*, 2017).In the current study, LPO level was increased in STZ-induced diabetic rats in comparison with control group. The highest decrease in the LPO level was found in diabetic rats treated with Glibenclamide, KKK (100, 200 and 300 mg) doses treatment also significantly decreased the level of LPO in pancreatic tissues according to varying doses. Figure 2 Data are expressed as mean ± SEM (n=6); $^{***}$p<0.001 vs control group. $^{###}$p<0.001, $^{@}$p<0.05, $^{@@}$p<0.01, $^{@@@}$p<0.001 vs diabetic group.

The diabetic rats showed (p<0.001) significant decrease in GPx, SOD and CAT activities and GSH content in comparison with control group. A significant increase in invivo antioxidant enzymes were noted in Glibenclamide treated rats when compared with STZ induced rats, similar to control group. Katakakhadiradi kashayam doses (100, 200 and 300 mg/kg) was found to be significantly effective in normalizing the antioxidants in diabetic condition but higher dose intake of this kashayam prove to be better compared with other doses. Figure 3 Data are expressed as mean ± SEM (n=6); $^{***}$p<0.001 vs control group. $^{###}$p<0.001, $^{@}$p<0.05, $^{@@}$p<0.01, $^{@@@}$p<0.001 vs diabetic group.

Figure 4 Data are expressed as mean ± SEM (n=6); ***p<0.001 vs control group. ###p<0.001, @p<0.05, @@p<0.01, @@@p<0.001 vs diabetic group.

The variation in SOD and GPX activities could be because of excessive consumption in the autoxidation procedure and increased excretion from the pancreatic tissue. The reduction in SOD activity may result in increased superoxide radical level which causes the inactivation of GPx activity (Panahi *et al.*, 2017).GPx catalyses low lipid peroxides formation by utilizing GSH, $H_2O_2$ is also being low released upon GPx activity by increased utilization of GSH. The high utilisation of GSH impairs the positive effect of antioxidant enzymes in pancreatic tissue. The free radicals scavenging activity of katakakhadiradi kashayam in comparison with ascorbic acid shows their abilities to decrease the hyperglycemic levels in diabetic patients and their capacities to hinder diabetes mellitus type 2 (Jessica *et al.*, 2017). Hence, it has been understood that the mechanism of antihyperglycemic action could be because of the antioxidant action of this kashayam. Most of the plants present in Katakakhadiradi kashayam have antidiabetic and antioxidant activities thus, effective in diabetes mellitus.

Many people in the society started using ayurvedic formulation for their life long, due to the safety and escape from the pharmacological drugs adverse effects which are sold in the market. Various phytoconstituents of katakakhadiradi kashayam has anti antipyretic, inflammatory, analgesic, antiemetic, hepatoprotective, antiarthritic, anticonvulsant, antioxidant, antidiabetic, anticancer, hypolipi-

demic, antioxidant, antibacterial, cytotoxic and apoptotic activities (Mohammad *et al.*, 2012). Most of the plants present in Katakakhadiradi kashayam have antidiabetic and antioxidant effects hence, treat or prevent this diseases with no toxicity and gives strength to affected tissues.[6]The result of the study showed that Katakakhadiradi kashayam could be used to improve management of diabetic condition which supports the medicinal intake of human beings.

## CONCLUSION

The Katakakhadiradi kashayam possesses strong hypoglycemic effect and also decreases oxidative stress in pancreatic tissue caused by streptozotocin indicating that these formulations inhibit oxidative damage by enhancing the tissue antioxidants. The present study result concluded that Katakakhadiradi kashayam is practically a safe herbal formulation can be used in the management of diabetes mellitus.

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### Conflict of Interest

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

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