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Hypoglycemic and hypocholesterolemic activities of the aqueous preparation of *Kalanchoe pinnata* leaves in streptozotocin-induced diabetic rats

Nikhil Menon, Jean Sparks, Felix Omoruyi*

Department of Life Sciences, Texas A&M University-Corpus Christi, Texas, USA

PEER REVIEW

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Professor Viroj Wiwanitkit, M.D., Visiting professor, Hainan Medical University, China; visiting professor, University of Nis, Serbia; adjunct professor, Joseph Ayobabalola University, Nigeria; honorary professor, Dr DY Patil Medical University, Nigeria; professor, senior expert, Surin Rajabhat University, Thailand.
Tel: 66892348832
E-mail: wviroj@yahoo.com

Comments

The report on animal model study with diabetes mellitus and the test regimen is on a local tropical species from Africa. The work is a good and standard study on ethnopharmacology. New information can be derived and this is useful for further drug research and development. In addition, the information from this work can add up the database on pharmacology, ethnopharmacology and diabetology. Further citation can be expected.
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ABSTRACT

Objective: To evaluate the hypoglycemic and hypocholesterolemic activities of the aqueous preparation of *Kalanchoe pinnata (K. pinnata)* leaves in streptozotocin-induced diabetic rats.

Methods: Diabetes mellitus was induced in rats by a single administration of streptozotocin (60 mg/Kg). Diabetic rats were then treated with aqueous *K. pinnata* for 30 d. Serum glucose, proteins, lipid composition, liver and kidney function indices, inflammatory markers, and key enzymes of hepatic carbohydrate and lipid metabolism were determined.

Results: The untreated and treated diabetic groups lost weight and consumed less food compared to the normal group. We noted 37.9% decrease in fasting blood glucose in the treated diabetic group compared to 13.2% and 17.0% increases in normal and untreated diabetic groups respectively. Serum cholesterol and triglyceride levels were significantly (P<0.05) reduced in the treated diabetic group compared to the untreated diabetic group. Blood urea nitrogen was significantly (P<0.05) elevated in the untreated and treated diabetic groups compared to the normal group. Serum alkaline phosphatase and hepatic pyruvate kinase activities were significantly (P<0.05) elevated in the treated diabetic group. Serum albumin level was significantly (P<0.05) reduced in the untreated diabetic group. Serum IL-6 was significantly (P<0.05) depressed in the treated diabetic group.

Conclusions: The observed decrease in body weight, blood glucose and cholesterol level suggests that the aqueous *K. pinnata* preparation consumption may be beneficial in the management of diabetes mellitus. The observed adverse effect on alkaline phosphatase activity may be due to the combined effect of streptozotocin-induced diabetes and *K. pinnata* preparation administration.

KEYWORDS

Kalanchoe pinnata, Diabetes, Streptozotocin, Hypoglycemic, Hypocholesterolemic

1. Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. This metabolic disorder includes alteration in carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and/or insulin action. The World Health Organization estimates that more than 347 million people worldwide have the disease. According to new estimates from the Centers for Disease Control and Prevention, about 26 million Americans have diabetes. Currently, there is no cure for the disease. Millions of people all over the world have resorted to the use of medicinal plants for the management of the disease due

*Corresponding author: Felix Omoruyi, Department of Life Sciences, Texas A&M University-Corpus Christi, Center for the Sciences, 130B, 6300 Ocean Drive, Unit 5802, Corpus Christi, TX 78412, USA.
Tel: 361-825-2473
Fax: 361-825-2135
E-mail: felix.omoruyi@tamucc.edu
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to the rising cost of orthodox treatment and associated side effects. Most of these medicinal plants are used in developing countries for the treatment of diabetes, especially in the underprivileged populations. Since diabetes is a global disease, more than 800 plants around the world have been identified as possible treatment options. Unfortunately, the use of many medicinal plant supplements in the management of diabetes and other diseases lack scientific backing. For example, doses and side effects of many medicinal plant preparations are not known. This makes herbal medicine a much riskier alternative to modern medicine. *Kalanchoe pinnata* (*K. pinnata*) is a medicinal plant found in South America, India, and the Caribbean. The plant has tall hollow stems, dark green scalloped leaves with red edges and produces dark bell-like pendulous flowers. The smaller reddish leaflets on the edges of the leaves are forming vegetative buds that are capable of producing individual plants on their own[1]. Aqueous preparations of plant leaves and roots are traditionally used for the treatment of a wide range of diseases in many parts of the world, including diabetes[2]. *K. pinnata* leaves have been reported to contain flavonoids, polyphenols, triterpenoids of β-amyrin structure, phytosterols *etc*[3,4]. The chemical constituents of the plant (flavonoids, polyphenols, triterpenoids and phytosterols) are speculated to account for the antinociceptive, antiinflammatory and antidiabetic activities of the herb's leaf aqueous extract[5]. However, in this study, we evaluated the hypoglycemic and hypocholesterolemic activities of the aqueous preparation of *K. pinnata* in streptozotocin-induced diabetic rats.

2. Materials and methods

2.1. Aqueous preparation of K. pinnata

A suitable weight of the plant leaves [the weight of the plant leaves was based on the weight of rats per cage to simulate the dose that is traditionally used in the management of diabetes (3 mature leaves ~9.96 g/70 kg body weight or about 0.14 g/kg body weight)] was homogenized in deionized water daily.

2.2. Induction of diabetes

Two of three groups received a single injection of streptozotocin (Sigma-Aldrich Co., St. Louis, USA, 60 mg/kg body weight in 0.05 mol/L citrate buffer, pH 4.5) intraperitoneally to induce diabetes. The third group (normal group) was injected intraperitoneally with an equivalent amount of buffer (0.05 mol/L citrate buffer, pH 4.5).

2.3. Experimental design

Eighteen adult Sprague rats were assigned by weight into three groups for a 30 d study [6 rats per group, average body weight (297.28±15.17) g]. The groups were composed as follows: healthy rats receiving deionized water (normal group); diabetic control rats administered deionized water (diabetic group); diabetic rats administered aqueous preparation of *K. pinnata* orally (3 mature leaves ~9.96 g/70 kg body weight or about 0.14 g/kg body weight, traditionally used in the management of diabetes, treated diabetic group). Rats were housed in cages with solid flooring covered with a bedding material. The cages were cleaned daily. Approval for the study was obtained after review of the protocol by the Institutional Animal Care and Use Committee of the Institute of Biosciences and Technology, Texas A&M Health Sciences Center, Houston with protocol number 13001. Diabetes was confirmed (with fasting blood glucose levels >240 mg/dL in the streptozotocin treated rats) by pricking the rat tail for a drop of blood for glucose determination after an overnight fast using a strip operated blood glucose meter (Bayer Contour Blood Glucose Monitoring System) on Day 8 after streptozotocin injection. Rats in all groups were fed a normal rat diet. Body weight change and total food intake were recorded weekly. Animals were euthanized by decapitation on Day 30 after commencement of the feeding trial excluding the 8 d period for the development of the animal model of the disease which all the rats were administered deionized water. Blood and organ samples were collected for assays.

2.4. Biochemical evaluation

Serum glucose, lipid profile, total protein, albumin, uric acid and creatinine levels, alkaline phosphatase, alanine and aspartate amino transferase activities were measured using reagent kits for Sirrus Clinical Chemistry Analyzer from Stanbio Laboratory, Boerne, TX, USA. The levels of interleukin-1beta (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in the serum were run in duplicate using commercial ELISA kits for rats (catalog numbers ER2IL1B, ER3IL6 and ER3TNFA respectively, according to the manufacturers' instructions (Thermo Scientific/Pierce Biotechnology, Rockford, IL, USA) and Bio-Rad Microplate reader. The results are expressed in g/L. Liver samples were weighed and homogenized with suitable buffer [60 mmol/L sucrose, 220 mmol/L mannitol, 10 mmol/L Tris-HCl (pH 7.4) containing 1 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L dithiothreitol 1:10 w/v]. Homogenates were centrifuged for 20 min at 5708 r/min and the supernatant used as the source of metabolic enzymes. Metabolic enzyme activities in the liver were determined by measuring the change in extinction due to nicotinamide adenine dinucleotide phosphate ($NADP^+$) reduction or reduced form of nicotinamide adenine dinucleotide oxidation[6].

2.5. Statistical analysis

Results are presented as mean±SEM, n=6. ANOVA was used to test for differences among the groups. *Post hoc* analysis was carried out using the Duncan's multiple range test to test for significant difference among the means (P<0.05).

3. Results

Figure 1 shows body weight changes and food intake in diabetic rats administered aqueous preparation of *K. pinnata*. There was a decreasing trend in the average food intake among the groups (normal group>diabetic group>treated diabetic group). The treated diabetic group consumed less food compared to the other groups. While the normal group gained weight, the treated diabetic group lost more weight compared to the diabetic group. We noted a decrease (37.9%) in fasting blood glucose in the treated diabetic group compared to increases in normal (13.2%) and diabetic (17.0%) groups.

Figure 2 shows serum lipid profile in diabetic rats administered aqueous preparation of *K. pinnata*. There was a significant (P<0.05) elevation in triglyceride level in the diabetic group, which was reduced towards normal level by the treatment. Total cholesterol level was also elevated in the diabetic group and there was a decreasing trend towards the normal group by the treatment. Additionally, high density lipoprotein (HDL) cholesterol was significantly (P<0.05) reduced in both diabetic and treated diabetic groups compared to the normal group.

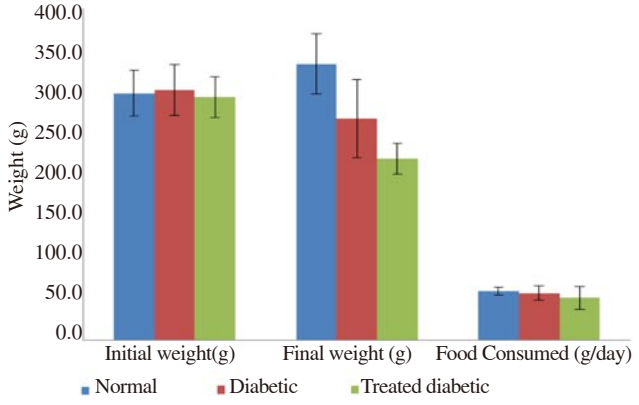

Figure 1. Body weight changes and food intake in diabetic rats administered aqueous preparation of *K. pinnata*.
Values were not significantly different among the groups (P>0.05).

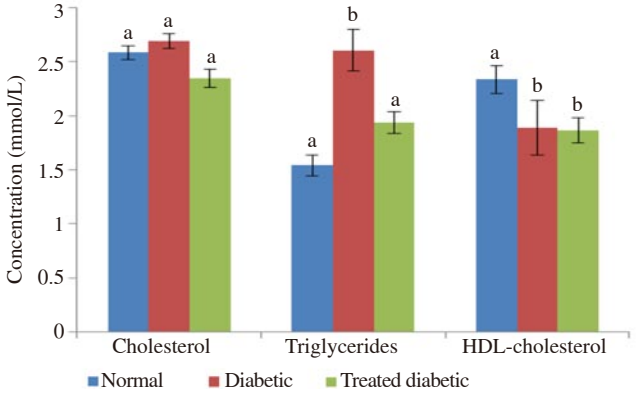

Figure 2. Serum lipid profile in diabetic rats administered aqueous preparation of *K. pinnata*.
Figures that share different letter superscripts are significantly different (P<0.05).

Figure 3 shows serum protein profile in diabetic rats administered aqueous preparation of *K. pinnata*. There was a significant (P<0.05) decrease in serum albumin level in the untreated diabetic group compared to the normal group. We observed increasing trend in albumin level in the treated diabetic group towards the normal group. The levels of total protein and globulin were not significantly altered among the groups. Similarly, albumin:globulin ratio was not significantly (P>0.05) altered among the groups (Figure 4).

Figure 5 shows liver function enzymes in the serum of diabetic rats administered aqueous preparation of *K. pinnata*. Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were not significantly altered among the groups. However, there was a significant (P<0.05) increase in serum alkaline phosphatase (ALP) activity in the diabetic and diabetic treated groups compared to the normal group.

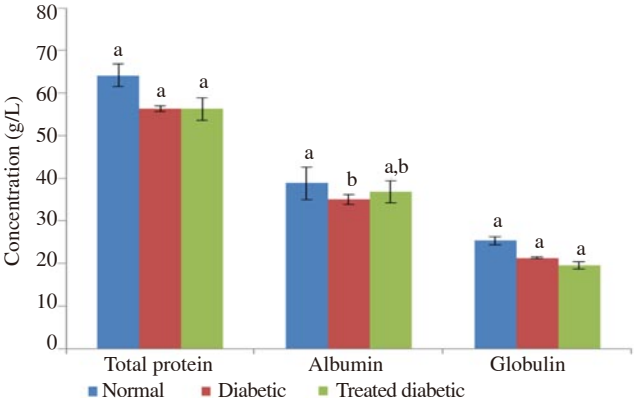

Figure 3. Serum protein profile in diabetic rats administered aqueous preparation of *K. pinnata*.
Figures that share different letter superscripts are significantly different (P<0.05).

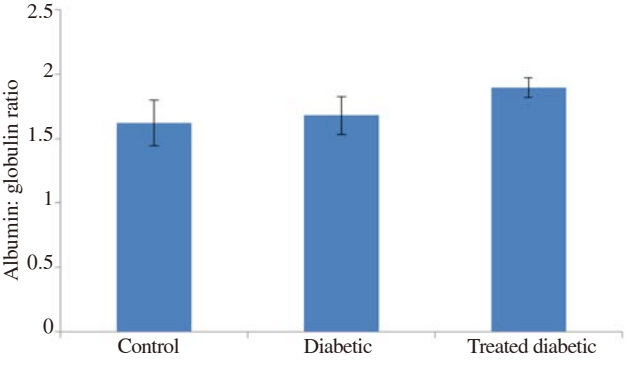

Figure 4. Albumin:globulin ratio in diabetic rats administered aqueous preparation of *K. pinnata*.
Values were not significantly different among the groups (P>0.05).

Figure 6 shows metabolic enzyme activities in the liver of diabetic rats administered aqueous preparation of *K. pinnata*. Hepatic glucose-6-phosphate dehydrogenase (G6PD), malic enzyme and $NADP^+$-isocitrate dehydrogenase activities were not significantly (P>0.05) altered among the groups. However, we noted a slight depression in hepatic G6PD activity in the treated diabetic group compared to the other groups. There was a significant (P<0.05) increase in pyruvate kinase activity in the treated diabetic group compared to the diabetic group.

Table 1 shows kidney function parameters in the serum of rats administered aqueous preparation of *K. pinnata*. There was a significant (P<0.05) increase in blood urea nitrogen (BUN) in the

diabetic and treated diabetic groups compared to the normal group. However, serum creatinine and uric acid levels among the groups were not significantly (P>0.05) altered.

Figure 7 shows serum interleukin levels in diabetic rats administered aqueous preparation of *K. pinnata*. We noted a significant (P<0.05) decrease in serum IL-6 levels in the treated diabetic group towards the normal group. There was a non-significant elevation of serum TNF-α levels in the treated diabetic group compared to the diabetic group. IL-1β level was elevated in the diabetic and treated diabetic groups compared to the normal group.

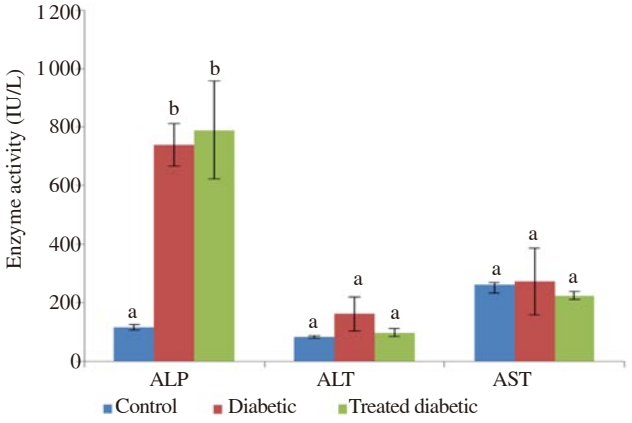

Figure 5. Liver function enzymes in the serum of diabetic rats administered aqueous preparation of *K. pinnata.*

Figures that share different letter superscripts are significantly different (P<0.05).

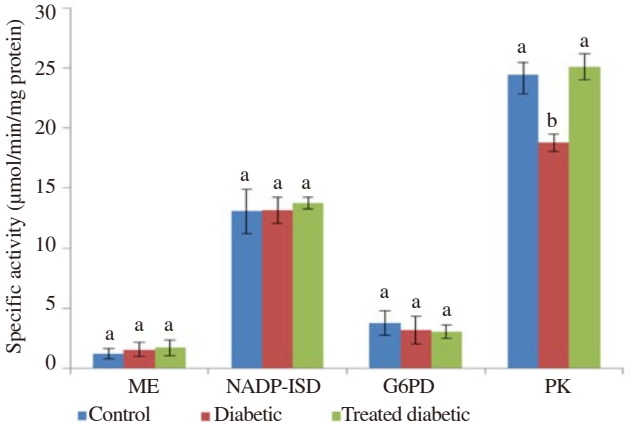

Figure 6. Metabolic enzyme activities in the liver of diabetic rats administered aqueous preparation of *K. pinnata.*

Figures that share different letter superscripts are significantly different (P<0.05). G6PD: Glucose-6-phosphate dehydrogenase; ME: Malic enzyme; NADP-ISD: $NADP^+$-isocitrate dehydrogenase; PK: Pyruvate kinase.

Table 1

Kidney function parameters in diabetic rats administered aqueous preparation of *K. pinnata*.

Groups	Uric acid (μmol/L)	Creatinine (μmol/L)	BUN (mmol/L)
Normal	91.48±24.32[a]	32.41±2.95[a]	7.56±0.27[a]
Diabetic	77.22±42.83[a]	26.52±3.95[a]	15.98±3.52[b]
Treated diabetic	112.86±59.40[a]	19.89±8.37[a]	20.17±3.08[b]

Values that share different letter superscripts vertically are significantly different (P<0.05).

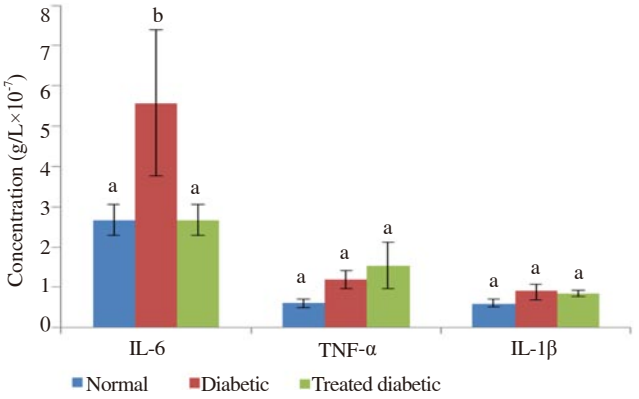

Figure 7. Interleukin levels in the serum of diabetic rats administered aqueous preparation of *K. pinnata.*

Figures that share different letter superscripts are significantly different (P<0.05).

4. Discussion

The results of this laboratory animal study show that aqueous preparation of *K. pinnata* possesses anti-diabetic activities in streptozotocin-induced diabetic rats. There was a decreasing trend in the average food intake among the groups (normal group>diabetic group>treated diabetic group). The untreated and treated groups lost weight and consumed less food compared to the normal control group. However, the diabetic treated group's loss in weight was more evident than the diabetic control group. Earlier study on blueberry extract with similar bioactive compounds also showed reduced food intake and body weight in rats due to its high satiating effects. However, the specific underlying mechanism was not presented[7]. It has also been reported that blueberries demonstrated antioxidant effects of reducing the accumulation of fat in aortas and livers of animal models, and reducing cardiovascular risk in obese individuals[8-11]. These biological effects are believed to be produced by phytosterols, phenolics and other bioactive compounds present in blueberries as *K. pinnata* aqueous preparation[5,12]. Since the leaves of *K. pinnata* are known to contain flavonoids, polyphenols, triterpenoids and phytosterols, it is generally believed that these chemical constituents might be responsible for the observed hypoglycemic activity of the herb[5]. Patterwar also reported that the plant could be beneficial in the management of diabetes because of the plant's rich source of zinc[13]. An earlier report showed that pharmacological doses of zinc supplementation of animals and humans ameliorate glycemic control in diabetes[14]. Zinc is believed to exert insulin-like effects by supporting the signal transduction of insulin and by reducing the production of cytokines, which lead to beta-cell death during the inflammatory process in the pancreas disease[14]. The observed decrease in serum, total cholesterol level in the treated diabetic group may also be due to the chemical constituents of the *K. pinnata* preparation. For example, it has been suggested that the ability of phytosterols to lower serum total cholesterol is due to the similarity of their chemical structures. They displace intestinal cholesterol with bile salt micelles and compete for absorption from the border brush, thus by suppressing the absorption of dietary and biliary cholesterol and upregulation of the enterocyte

adenosine triphosphate-binding cassette transport proteins[15,16]. Similarly, Ho *et al*., showed that feeding 0.72% phytosterol-containing milk powder lowered total cholesterol by 31% and low-density lipoprotein-cholesterol by 52% in hypercholesterolemic hamsters[17]. Also, flavone, a class of flavonoids, has been reported to induce lipid lowering action in hyperlipidemic rats[18]. Gwon *et al*. suggested that flavones may be one of the candidates for an active component in *Zanthoxylum piperitum* extract as in *K. pinnata* aqueous preparation[19]. In their study, sterol regulatory element binding protein 1 was regulated by flavones, which led to down regulation of transcription factors and fatty acid synthase. They suggested that the inhibitory effect of *Zanthoxylum piperitum* extract on high fat diet-induced obesity may be partially attributed to flavones, which could also explain the decrease in body weight in our study. In addition, several systemic peptides have been shown to regulate appetite and body weight. We suggest further studies on the potential role of *K. pinnata* aqueous preparation on vital systemic peptides involved in the regulation of appetite and body weight. Previous studies of various natural extracts containing polyphenols were shown to decrease lipid accumulation and stimulate lipolysis in preadipocytes and adipocytes[20,21]. Elevated triglyceride level and reduced HDL levels are conducive to the development of atherosclerosis and increases the risk of cardiovascular disease[22]. In the diabetic state, there is a defect in the metabolism of stored triglycerides by adipose tissue resulting in elevated transport of free fatty acids to the liver, which triggers the overproduction of large very low-density lipoprotein fragments and triglycerides[23]. These result in secondary abnormalities of low HDL-cholesterol and increased low density lipoprotein (LDL)[24]. Elevated level of LDL cholesterol is subject to oxidative modification and transformation into foam cells, which accumulate in the form of fatty streaks and fibrofatty plaques that lead to the development of atherosclerosis[23]. Although the treatment was able to decrease the triglyceride level which may reduce cardiovascular risk, and the non-restoration of HDL-cholesterol level towards the normal control group may be a drawback in the use of the preparation for the management of diabetes. However, the evaluation of the entire lipid profile which includes LDL, very low-density lipoprotein and lipid moieties are required to ascertain whether the tissues are taking up excess lipids or improving fuel utilization. The liver is the main site of synthesis of plasma proteins, especially albumin. Changes in serum total proteins or the ratio of albumin and globulin may be indicative of hepatic dysfunction. These changes in plasma proteins usually do not appear except in chronic or severe liver dysfunction that may be evidenced by a decrease in albumin and an increase in globulin concentration. The untreated diabetic group showed a significant decrease in the albumin level comparable to the normal control group. However, there was an increasing trend in the albumin level in the diabetic treated towards the normal control group. The liver enzymes, ALT, AST and ALP are cellular enzymes that are present in low concentrations in serum under normal conditions. Significant increases in serum concentrations result from either increased synthesis of the enzyme or increased rate of leakage from damaged cells[25]. Serum ALT and AST levels are clinically and toxicologically important indicators of tissue damage caused by toxicants or disease conditions[26]. Changes in detectable liver enzyme activity before physical symptoms of tissue damage are usually apparent. Our data on serum proteins and liver function enzymes showed that the consumption of *K. pinnata* preparation may protect against liver damage associated with diabetes mellitus. Although the observed increase in serum alkaline phosphatase activity may be indicative of tissue damage in the diabetic treated group. However, it can be highly nonspecific in terms of organ function. Further studies are needed on the effect of the treatment on alkaline phosphatase activity in diabetes mellitus.

One of the regulatory reactions of glycolysis is catalyzed by pyruvate kinase, while one key reaction of the pentose phosphate pathways is catalyzed by G6PD. This latter reaction generates reduced nicotinamide adenine dinucleotide phosphate (NADPH) for the synthesis of steroids and fatty acids. Malic enzyme and NADP-ISD similarly generate NADPH for reductive biosynthesis. The observed significant (P<0.05) increase in pyruvate kinase activity in the treated diabetic group may suggest heightened glucose metabolism due to consumption of the aqueous *K. pinnata* preparation which may account for the hypoglycemic activity. The slight depression in hepatic G6PD activity in the treated diabetic group may be indicative of a depressed pentose phosphate pathway. This pathway generates NADPH for the synthesis of steroids and fatty acids, and could partly explain the observed hypocholesterolemic activity of *K. pinnata* preparation consumption in diabetes mellitus.

The observed significant (P<0.05) increase of BUN in the diabetic and diabetic treated groups compared to the control group may be indicative of renal insufficiency or dehydration and this is usually coupled with an increase in creatinine level, but this was not the case in this study. Both BUN and creatinine are valuable screening tests in evaluating renal disease. However, creatinine is a more reliable indicator of renal function than BUN because BUN is more likely to be affected by dietary and physiologic conditions that are not related to renal function.

Systemic IL-1β, TNF-α, and IL-6 levels have been found to be elevated during aging and diabetes[27]. Proinflammatory cytokines such as TNF-α and IL-1β also participate in the pathogenesis of atherosclerosis by inducing E-selectin, intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 expression in endothelial cells[28]. Kong *et al*. reported that IL-1β and TNF-α are two important proinflammatory cytokines in the initiation and development of atherosclerosis[29]. The elevated levels of these interleukins (IL-1β and TNF-α) in our study suggest that the use of *K. pinnata* preparation in the management of diabetes mellitus may not be effective in ameliorating the potential for the development of atherosclerosis. Our data show that another proinflammatory cytokine (IL-6) that is up-regulated in diabetes was significantly depressed by the administration of aqueous preparation. The level of IL-6 has been shown to be elevated during the acute phase response, which could be triggered by diabetes and subsequently lead to the release of effector molecules that might cause endothelial

dysfunction leading to atherosclerosis[30,31]. We hypothesize that the observed significant down-regulation of IL-6 in the treated diabetic group ameliorates the adverse effects associated with the up-regulation of IL-1β and TNF-α, which may account for our observation that *K. pinnata* preparation may protect against cardiovascular risk associated with diabetes mellitus.

Overall, the aqueous preparation of *K. pinnata* demonstrated a decrease in body weight, and hypoglycemic and hypocholesterolemic activities which are beneficial in the management of diabetes. The decrease in blood glucose may be due to the increased glycolytic pathway in the liver as shown by the elevated pyruvate kinase activity. While the elevation of BUN could be a cause for concern, it is not the most sensitive indicator of renal damage and could be elevated due to a number of unrelated factors. The inflammatory cytokine (IL-6) normally up-regulated in diabetes was significantly depressed by the aqueous preparation. Although the consumption of the aqueous preparation of *K. pinnata* may accrue some benefits in the management of diabetes, further studies are needed to evaluate the long-term effects of *K. pinnata* preparation versus a positive control drug currently used in the treatment of diabetes mellitus on serum ALP activity and BUN level in streptozotocin-induced diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

This is an animal study on the pathogenesis of diabetes mellitus. It is a kind of ethnopharmacology of a locally available species in Africa. The work can be a good data for further study.

Research frontiers

This is a good animal model study on a tropical species that can be applicable used as a local wisdom regimen against diabetes mellitus. The information is new and interesting. Also, the result can be useful in diabetology in the future.

Related reports

There are some related reports but not on this specific studied species. The work remains its originality and has new data.

Innovations and breakthroughs

This work can be further applied in the future biomedical study on this tropically available species. The work represents a good example of ethnopharmacology investigation and the data is applicable for diabetology.

Applications

The work can be good basic information for further diabetology research. Also, the study represents good ethnopharmacology that can be further referenced in the related field.

Peer review

The report on animal model study with diabetes mellitus and the test regimen is on a local tropical species from Africa. The work is a good and standard study on ethnopharmacology. New information can be derived and this is useful for further drug research and development. In addition, the information from this work can add up the database on pharmacology, ethnopharmacology and diabetology. Further citation can be expected.

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