

Oxidative Stress Parameters and Erythrocyte Membrane Adenosine Triphosphatase Activities in Streptozotocin-induced Diabetic Rats Administered Aqueous Preparation of *Kalanchoe Pinnata* Leaves

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ABSTRACT

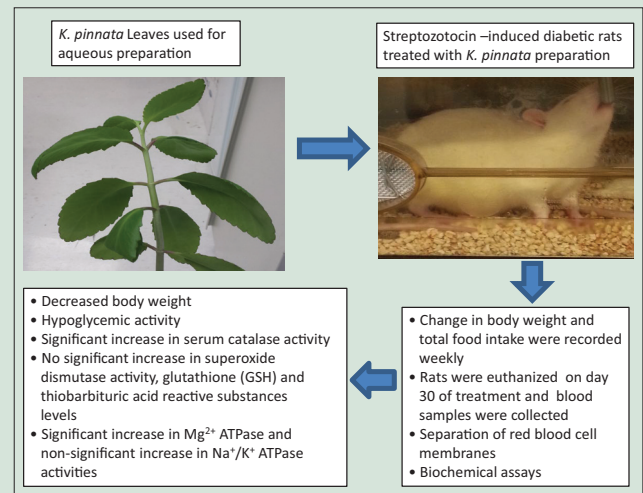
Background: Diabetes mellitus is a chronic metabolic disease that according to the World Health Organization affects more than 382 million people. The rise in diabetes mellitus coupled with the lack of an effective treatment has led many to investigate medicinal plants to identify a viable alternative. **Objective:** To evaluate red blood cell (RBC) membrane adenosine triphosphatase (ATPase) activities and antioxidant levels in streptozotocin-induced diabetic rats administered aqueous preparation of *Kalanchoe pinnata* leaves. **Materials and 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* preparation (three mature leaves ~ 9.96 g/70 kg body weight or about 0.14 g/kg body weight/day) for 30 days. Serum glucose, RBC membrane ATPase activities, and antioxidant levels were determined. **Results:** We noted weight loss and reduced food consumption in the treated diabetic group. Serum glucose levels were reduced in the treated diabetic group compared to the other groups. Superoxide dismutase activity and glutathione levels were not significantly elevated in the treated group compared to the diabetic group. However, serum catalase activity was significantly ($P < 0.05$) increased in the treated diabetic group compared to the other groups. Serum thiobarbituric acid reactive substances were not significantly altered among the groups. There was a significant ($P < 0.05$) increase in Mg^{2+} ATPase activity and a nonsignificant increase in Na^+/K^+ ATPase activity in the RBC membrane of the treated diabetic group compared to the diabetic group. **Conclusion:** The consumption of aqueous preparation of *K. pinnata* may accrue benefits in the management of diabetes by lowering oxidative stress often associated with the disease and improving the availability of cellular magnesium through an increase in the magnesium ATPase pump in the RBC membrane for increased cellular metabolism of glucose through the glycolytic pathway. **Key words:** Antioxidant enzymes, diabetes, *Kalanchoe pinnata*, red blood cell membrane adenosine triphosphatases

SUMMARY

- We noted weight loss and reduced food consumption in the diabetic rats treated with *K. pinnata* preparation
- Serum glucose levels were reduced in diabetic rats treated with *K. pinnata* preparation
- Serum catalase activity was significantly ($P < 0.05$) increased in diabetic rats

treated with *K. pinnata* preparation

- We also noted a significant ($P < 0.05$) increase in Mg^{2+} ATPase activity in the RBC membranes of diabetic rats treated with *K. pinnata* preparation
- Overall, the consumption of aqueous preparation of *K. pinnata* lowered oxidative stress often associated with diabetes and improved availability of cellular magnesium through an increase in magnesium ATPase pump in the RBC membrane.



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INTRODUCTION

Diabetes is a metabolic syndrome characterized by hyperglycemia resulting from alterations of insulin secretion or action. The World Health Organization estimates that more than 382 million people worldwide have the disease, and approximately 24.4 million of those patients are Americans.^[1] Currently, there is no cure for diabetes. Millions of people all over the world have resorted to the use of medicinal plants for the management of the disease due to the rising cost of orthodox treatment and the 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

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and other diseases lack scientific backing. These treatments also lack standardization, and the optimal doses and side effects of many of these medicinal plant preparations are unknown. This makes herbal medicine a much riskier alternative to modern medicine. *Kalanchoe pinnata* is one such 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.^[2] The smaller reddish leaflets on the edges of the leaves are rooting vegetative buds that are capable of producing individual plants on their own.^[2] Aqueous preparations of the plant leaves and roots are traditionally used for the treatment of a wide range of diseases in many parts of the world, including diabetes.^[3] *K. pinnata* leaves have been reported to contain flavonoids, polyphenols, triterpenoids of β -amyrin structure, phytosterols, and so on.^[4,5] The chemical constituents (flavonoids, polyphenols, triterpenoids, and phytosterols) of the plant are speculated to account for the antinociceptive, anti-inflammatory and antidiabetic activities of the herb's leaf aqueous preparation.^[6] In this study, we evaluated the antidiabetic effect of aqueous *K. pinnata* preparation by specifically evaluating its effect on the antioxidant defense system and red blood cell (RBC) membrane adenosine triphosphatase (ATPases) in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Animals and induction of diabetes

Eighteen adult Sprague–Dawley rats were assigned by weight into three groups for a 30 day study (six rats per group, average body weight 297.28 ± 15.17 g). The groups were composed as follows: Healthy rats receiving deionized water (normal); diabetic rats administered deionized water (diabetic); diabetic rats administered aqueous preparation of *K. pinnata* [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)]. Two of the three groups received a single injection of streptozotocin (Sigma-Aldrich, 60 mg/kg body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally to induce diabetes. The third group, the normal control group was injected intraperitoneally with an equivalent amount of buffer (0.05 M-citrate buffer, pH 4.5). Rats were housed in cages with solid flooring covered with a bedding material and allowed free access to food and aqueous preparation or deionized water. 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 University Health Sciences Center, Houston with protocol number 13001. All institutional and national guidelines for the care and use of laboratory animals were followed. 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. Body weight change and total food intake were recorded weekly. Animals were euthanized by decapitation on day 30 after the commencement of the feeding trial excluding the 8 day period for the development of the animal model of the disease when all the rats were administered deionized water. Blood was collected for biochemical assays.

Processing of red blood cell

RBC membranes were prepared from the blood samples collected in ethylenediaminetetraacetic acid containing tubes. The blood samples were centrifuged at $3000 \times g$ for 20 min at 4°C . The packed cells were washed 3 times with 0.3 M Tris-HCl buffer, pH 7.4 (isotonic). An aliquot of 1.0 mL washed cells was lysed using 9.0 mL of 0.015 M Tris-HCl

buffer, pH 7.2 (hypotonic). The lysed cells were then centrifuged for 30 min at $15,000 \times g$.^[7] The supernatants (hemolysates) were saved for the lipid peroxidation assay, and the pellet was repeatedly washed with hypotonic Tris-HCl buffer until a clear pale pink or colorless supernatant was obtained. The resulting erythrocyte membrane was suspended in 0.01 M Tris-HCl buffer, pH 7.4, and used for the determination of ATPase activities.

Biochemical assays

Serum glucose was measured using reagent kit from Stanbio, USA. ATPase activity was determined by measuring the liberation of inorganic phosphate following incubation with 3 mM disodium ATP and cations for the appropriate ATPase.^[8] Inorganic phosphate released in the ATPase assay was determined by the method of Fiske and Subbarow.^[9] Serum superoxide dismutase (SOD) activity was determined according to the method described by Marklund and Marklund.^[10] One unit of the enzyme was defined as the amount of the enzyme required for 50% inhibition of autoxidation of pyrogallol in 1 min. Serum catalase (CAT) activity was assayed by the method of Aebi and was determined as residual H_2O_2 after incubation with the enzyme.^[11] The serum reduced glutathione level was determined by the method described by Ellman in which the serum was mixed with 10% trichloroacetic acid and centrifuged.^[12] The supernatant was then treated with an appropriate amount of Ellman's reagent to yield a color that was measured spectrophotometrically. Lipid peroxidation was determined by the method of Genet *et al.*^[13] Values for thiobarbituric acid reactive substances are reported as malondialdehyde (MDA) and quantified using a molar extinction coefficient of 1.5×10^5 M/cm and expressed as mmol MDA/mg protein.

Statistical analysis

Results are presented as mean \pm standard error of the mean. Analysis of variance was used to test for differences among the groups. A *post-hoc* analysis was carried out using the Duncan's multiple range test to test for significant difference among the means ($P < 0.05$).

RESULTS

Figure 1 shows the changes in body weight and average food intake in rats administered aqueous preparation of *K. pinnata*. There was a decreasing trend in the average food intake among the groups (control $>$ diabetic $>$ treated diabetic). The treated diabetic group consumed less food compared to the other groups. While the normal control group gained weight, the treated diabetic group lost more weight compared to the diabetic control 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 the effect of *K. pinnata* preparation on RBC membrane ATPase activities in diabetic rats. Na^+/K^+ ATPase and Ca^{2+} ATPase activities were not significantly altered among the groups. We noted a significant ($P < 0.05$) increase in erythrocyte membrane Mg^{2+} ATPase activity in the treated diabetic group compared to the diabetic control group.

Table 1 shows the effect of aqueous *K. pinnata* preparation on the antioxidant status in the serum of diabetic rats. Serum SOD activity and reduced glutathione (GSH) level were not significantly increased in the treated diabetic group compared to the diabetic control group. Serum CAT activity was significantly ($P < 0.05$) increased in the treated diabetic group compared to the other groups. Serum thiobarbituric acid reactive substances (TBARS) levels were not significantly altered among the groups in this short-term study.

DISCUSSION

We have earlier reported the hypoglycemic and hypocholesterolemic activities of *K. pinnata* preparation consumption in streptozotocin-induced diabetes.^[14] This report provides data on the role of *K. pinnata* preparation consumption on lipid peroxidation, antioxidant enzymes and RBC membrane ATPase activities in streptozotocin-induced diabetic rats. Diabetic conditions have been shown to result in impaired antioxidant defenses, compromised mitochondrial function, and increased sorbitol and advanced glycation end products from glucose.^[15] Previous studies have shown that oxidative stress generated from hyperglycemia plays a particularly important role in the initiation of vascular diabetic complications, including retinopathy, cardiomyopathy, and nephropathy.^[16] SOD and CAT are antioxidant enzymes that convert toxic free radicals to water or other harmless compounds. Lipid peroxidation is intricately linked to antioxidant enzymes such as SOD and CAT. As a defense against reactive free radicals, the body produces antioxidant enzymes which help to prevent oxidative stress damage to tissues. The observed decreases in SOD and CAT activities and GSH levels in the untreated diabetic group is consistent with reports in the literature suggesting the disposition of diabetic tissues to damage by free radicals.^[17] Although, the TBARS levels were not significantly altered among the groups in this short-term study, the observed early increased SOD and CAT activities in diabetic rats treated with *K. pinnata* preparation may be indicative that the preparation might be effective in curtailing lipid peroxidation associated with the disease. This would spare tissue damage and prevent the development of diabetic complications.

Constant exposure to free radicals and high oxidative stress in diabetes have also been associated with erythrocyte structural damage.^[18] Lipid peroxidation alters the cellular structure of membrane-bound enzymes by changing phospholipids and fatty acid composition. Previous studies have reported reduced erythrocyte ATPase activity, specifically the

Na⁺/K⁺ ATPase in insulin-deficient conditions.^[19,20] In this study, we noted a nonsignificant decrease in Na⁺/K⁺ and Mg²⁺ ATPase activities and a nonsignificant increase in Ca²⁺ ATPase activity in the diabetic control group. However, in the diabetic group treated with aqueous *K. pinnata* preparation, there was a marginal increase in Na⁺/K⁺ activity and a significant ($P < 0.05$) increase in Mg²⁺ ATPase activity compared to the diabetic control group. Mg²⁺ ATPase activity in the erythrocyte membrane has been reported to reduce cellular calcium content, improve erythrocyte flexibility and reduce vascular complications.^[21] In fact, *in vitro* and *in vivo* studies have demonstrated that insulin may modulate the shift of magnesium from extracellular to intracellular spaces.^[22,23] Intracellular Mg²⁺ is a critical cofactor for several enzymes in carbohydrate metabolism because of its role as part of the activated Mg²⁺ ATP complex. The activated complex is responsible for the phosphorylation of all the rate limiting enzymes in the glycolytic pathway.^[24] Mg²⁺ deficiency may also result in disorders of tyrosine-kinase activity on the insulin receptor, leading to the development of postreceptor insulin resistance and decreased cellular glucose utilization.^[25] Hence, the observed significant increase in RBC membrane Mg²⁺ ATPase activity may suggest that the consumption of aqueous *K. pinnata* preparation could increase intracellular magnesium and subsequently improve rates of glycolytic activity.^[14] Adamson *et al.* also reported that the determination of ATPases, particularly Mg²⁺ ATPase and Na⁺/K⁺ ATPase, may provide an indirect measurement of insulin binding or level.^[26] We hypothesize that the consumption of *K. pinnata* preparation may act to increase peripheral insulin levels via extrapancreatic insulin elevation. It is also possible that

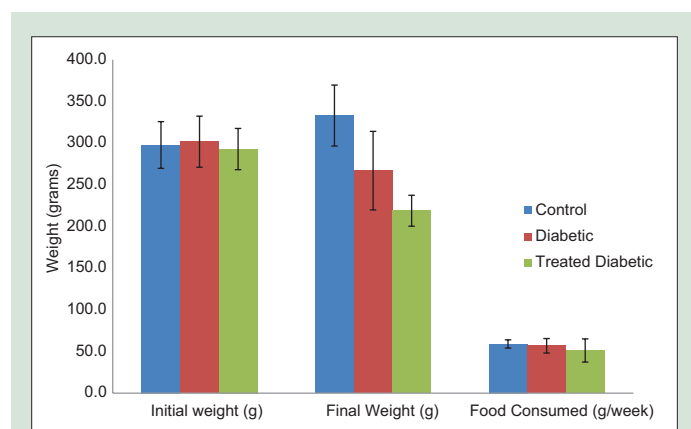


Figure 1: Body weight changes and food intake of diabetic rats administered aqueous preparation of *Kalanchoe pinnata*. Means \pm standard error of the mean, values were not significantly different among the groups ($P > 0.05$)

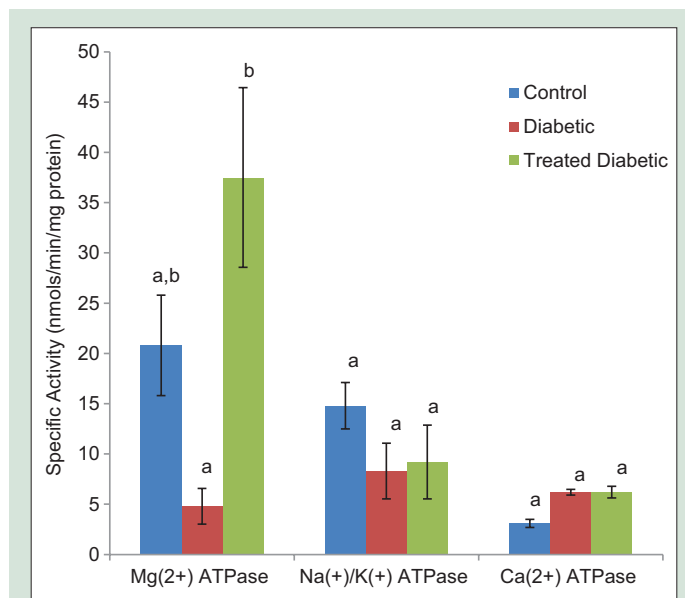


Figure 2: Effect of aqueous *Kalanchoe pinnata* preparation on red blood cell membrane adenosine triphosphatase activities in diabetic rats. Means \pm standard error of the mean, figures that share different letter superscripts are significantly different ($P < 0.05$)

Table 1: Effect of aqueous *Kalanchoe pinnata* preparation on the antioxidant status in the serum of diabetic rats

	SOD (U/mg protein/min)	GSH (μ M/mg protein)	CAT (mmol H ₂ O ₂ /mg protein/min)	TBARS (mmol MDA formed/mg protein)
Control	0.87 \pm 0.18 ^a	202.29 \pm 24.63 ^a	0.17 \pm 0.04 ^a	3.42 \pm 0.20 ^a
Diabetic	0.61 \pm 0.09 ^a	162.03 \pm 24.67 ^a	0.13 \pm 0.02 ^a	3.66 \pm 0.33 ^a
Treated diabetic	0.82 \pm 0.21 ^a	208.40 \pm 6.93 ^a	0.34 \pm 0.12 ^b	3.60 \pm 0.23 ^a

Means \pm SEM, values that share different letter superscripts vertically are significantly different ($P < 0.05$). SEM: Standard error of the mean; SOD: Superoxide dismutase; GSH: Reduced glutathione; CAT: Catalase; TBARS: Thiobarbituric acid reactive substances; MDA: Malondialdehyde

the preparation's active ingredient may modulate the insulin–insulin receptor complex with a resultant prolonged half-life of ligand–receptor complex, which could lead to sustained signaling as was reported for the AspB10 insulin analog.^[27] More investigations are needed to further isolate and identify the active component(s) of the preparation, as well as to investigate, the effects of these isolates on glucose-regulating hormones.

CONCLUSION

Overall, the administration of aqueous *K. pinnata* preparation demonstrated a decrease in body weight, and improved hypoglycemic activity. The decrease in blood glucose may be a factor in the reduction of lipid peroxidation and improvement in the antioxidant (SOD, CAT, and GSH) defense system. Improved RBC magnesium ATPase activity due to the treatment with aqueous *K. pinnata* preparation may be indicative of improved erythrocyte flexibility and increased cellular glucose utilization.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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