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Studies on the antidiabetic activity of *Ananas comosus* leaves in STZ induced diabetic rats

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ABSTRACT

Traditional medicines are originated from plants that do not form the constituents of routine diet. However, most of the medicinal plants have not received proper scientific scrutiny. Ananas comosus is one such plant, traditionally used for the treatment of various ailments. Hence, in the present study, an earnest attempt has been made to explore the antidiabetic and antioxidant properties of Ananas comosus leaves in STZ induced diabetic rats. The phytochemical screening of the Ananas comosus leaves extract indicated the presence of alkaloids, flavonoids, tannins, phytosterol, glycosides and phenols. The effect of oral administration of Ananas comosus leaves extract (300mg/kg b.w.) on the levels of biochemical parameters were determined in experimental groups of rats. The altered levels of biochemical parameters in the diabetic rats were significantly reverted back to near basal values upon treatment with the leaves extract. The levels of glycogen content, the activities of glycogen metabolizing enzymes were improved upon treatment with the leaves extract. The elevated activities of serum aminotransferases and alkaline phosphatase were reduced upon treatment with the extract. The increased levels of lipid peroxides in the plasma and pancreatic tissues of diabetic rats were normalized significantly by the administration of leaves extract. The activities of pancreatic enzymatic and the levels of plasma non-enzymatic antioxidants were markedly declined in the diabetic rats. Upon treatment with leaves extract to diabetic rats, the decreased levels were elevated to near normal values. The results of the study indicate that Ananas comosus leaves extract possesses significant antidiabetic activity which in turn is partially due to its antioxidant nature. The biologically active ingredients present in the Ananas comosus leaves extract may account for the observed pharmacological action.

Keywords: Diabetes mellitus; STZ; *Ananas comosus*; antidiabetic; antioxidant

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INTRODUCTION

Diabetes is a chronic metabolic disorder with devastating complications, mostly due to accelerated micro vascular and macro vascular disorders. Studies have demonstrated that tight control of blood glucose levels minimizes the complications of diabetes. Drugs play an important role in the treatment of diabetic complications. A rapidly expanding therapeutic armamentarium is now available to treat diabetic complications [1]. The pathophysiology involves insulin deficiency, interaction between genetic and environmental factors, insulin resistance, insulin sensitivity coupled with inadequate insulin response [2]. The global pattern is dominated by countries with large populations, and these data highlight the extent to which demographic changes in India, China and Brazil are likely to affect the total numbers with diabetes in the future [3].

The major aim of treatment of diabetes is to control hyperglycemia and its mediated complications. Rational therapy of diabetes requires the application of principles derived from current knowledge concerning both the nature of the particular type of diabetes and the mechanism of action, efficacy and safety of the available treatment regimens. Currently available oral hypoglycemic drugs for the treatment of diabetes such as sulphonylureas, biguanides, α-glycosidase inhibitors and thiazolidinediones are often associated with undesirable side effects or

diminution in response after prolonged use. Hence, the search continues for novel drugs with effective antidiabetic activity at low concentration without side effects.

In developing countries, traditional medicine is often the only accessible and affordable treatment available. Medicinal plants consist of components of therapeutic values and have been used as remedies for human diseases since antiquity. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-toxic, having no side-effects, easily available at affordable prices. There are several medicinal plants with potent antidiabetic activity. In the series of medicinal plants, one such medicinal plant that lack scientific scrutiny is *Ananas comosus.*

Ananas comosus (L.) MERRILL. (family Bromeliaceae), also, commonly named Pineapple, is a tropical fruit which grows in countries which are situated in the tropical and sub tropical regions. It is an herbaceous perinneal plant. For several thousand of years , superior types of pineapple were domesticated and distributed by native Indians throughout the tropics and subtropics [4, 5]. This plant is known for its folk medicinal utility, besides agricultural utilities such as the fruit for nutritional food.Total pineapple production worldwide is around 16-18 million tons [6, 7]. Sixty percent of fresh pineapple is edible [8]. It is composed of several nutrients which are essential for human health [9]. It has long pointed leaves and is 20-72 inch in length, usually needle tipped and bear sharp, up curved spines in the margins. Most of the leaves especially the leaves at the top of the plant most exposed to the sun are oriented at an angle to the sun and this arrangement helps to reduce leaf temperature and moisture loss. The successful dispersion of pineapple on a worldwide basis can be attributed to its ability to tolerate drought and the relative case with which vegetative propagules can establish under cultivated conditions [10]. Pineapple contains significant amounts of vitamins and it is considered as good source of essential trace elements [11, 12]. Bromelain, a proteolytic enzyme present in the fruits is reported to be responsible for its meat tenderizing properties and many of its medicinal properties [13].

Ananas comosus possess a wide array of pharmacological properties such as antibacterial activity [14], antihyperlipidemic activity [15], Antidysuria activity [16], antitumor activity [17] . In the absence of systematic studies in literature, the present study is aimed to evaluate the antidiabetic and antioxidant *Ananas comosus* leaves in STZ-induced experimental rats.

MATERIALS AND METHODS

Experimental Animals

Male albino Wistar rats (150-180 g) were purchased from TANUVAS, Madavaram, Chennai. The rats were housed in polypropylene cages lined with husk and kept in Animal house, Department of Biochemistry. It was renewed every 24 hours. The rats were fed with commercial pelleted rats chow (VRK Nutritional Solutions, Maharashtra, India) and had free access to water. The experimental rats were maintained in a controlled environment (12:12 hours light/dark cycle) and temperature (30 ± 2°C). The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines for the investigation of experimental pain in conscious rats. The rats were acclimatized for one week before initiating the experimental protocol.

Plant Material

The leaves of *Ananas comosus* were collected from Dindigul District, Tamilnadu. The leaves were identified and authenticated by a qualified taxonomist in the CAS in Botany, University of Madras for future reference.

Preparation of Plant extract

The leaves of *Ananas comosus* were dried at room temperature and powdered in an electrical grinder, which was then stored in an airtight container at 5°C until further use. The powdered leaves were delipidated with petroleum ether (60 - 80°C) for overnight. It was then filtered and soxhalation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40 – 50°C under reduced pressure. The yield was 26.2% w/w.

Preliminary Phytochemical Screening

The ethanolic extract of *Ananas comosus* leaves were subjected to preliminary phytochemical screening of various plant constituents [18, 19].

Induction of Diabetes Mellitus

Experimental diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (45 mg/kg b.w) dissolved in freshly prepared 0.1M of cold citrate buffer (pH 4.5) [20]. Since, STZ is capable of inducing fatal hypoglycemia due to massive pancreatic insulin release, the rats were provided with 10% glucose

solution after 6 h of STZ administration for the next 24 h to overcome drug induced hypoglycemia [21]. Neither death nor any other adverse effect was observed. After a week time, for the development and aggravation of diabetes, rats with moderate diabetes (i.e. fasting blood glucose concentration, >250 mg/dl) that exhibited hyperglycemia and glycosuria were selected for further experimentation.

Experimental Design

The rats were grouped into 4 groups, comprising of 6 rats in each group as follows:

Group I **:** Control rats
Group II **:** STZ induced diabetic Rats.
Group III **:** Diabetic rats treated with *Ananas comosus leaves* extract (300 mg/kg bw/day) in aqueous solution orally for 30 days.
Group IV **:** Diabetic rats treated with gliclazide (5mg/Kg body weight/day) in aqueous solution orally for 30 days.

During the experimental period, body weight and blood glucose levels of all the rats were determined at regular intervals. At the end of the experimental period, the rats were fasted over night, anaesthetized, and sacrificed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation respectively.

The liver and pancreatic tissues were dissected out and washed in ice-cold saline, which is then used for further experimental studies.

Preparation of tissue homogenate

The liver and pancreatic tissues were excised, rinsed in ice-cold saline. Known amount of the tissues were homogenized in Tris–HCl buffer (100 mM, pH 7.4) at 4°C, in a Potter–Elvehjem homogenizer with a Teflon pestle at 600 rpm for 3 min. The homogenate was then centrifuged at 12,000×g for 30 min at 4°C. The supernatant was collected as tissue homogenate, which was used to assay of various parameters. The protein content in the tissue homogenate was estimated by the method of Lowry et al (1951) [22]. A portion of wet liver tissue was used for the estimation of glycogen content [23]. The activities of glycogen synthase [24], glycogen phosphorylase [25] were assayed in liver tissues.

Oral Glucose Tolerance Test (OGTT)

At the end of the experimental period, fasting blood samples were taken from all the groups of rats. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after administration of glucose solution at a dosage of 2 g kg^{-1} body weight. All the blood samples were collected with EDTA for the estimation of glucose.

Biochemical parameters

Blood glucose level was estimated by the method of glucose oxidase/peroxidase method as described by Trinder (1969) using a commercial kit [26] (Span Diagnostic Chemicals, India) and urea [27] (Natelson et al., 1951). Glycosylated hemoglobin was estimated according to method of Nayak and Pattabiraman, (1981) [28]. Plasma was used for protein assay [22]. Urine sugar was detected using urine strip. Serum was used for the determination of creatinine [29] and uric acid [30]. Aspartate transaminase(AST), Alanine transaminase(ALT)and Alkaline phosphatase (ALP) were assayed [31, 32].

Assay of antioxidant status

The levels of lipid peroxides were determined in plasma and tissue homogenate [33, 34]. The activities of enzymatic antioxidants such as SOD [35], catalase [36] and GPx [37] were assayed in the tissue homogenate of control and experimental groups of rats. The levels of nonenzymatic antioxidants, vitamin C, vitamin E, and GSH were determined [38, 39, 40].

Statistical Analysis

The values were expressed as mean ± S.D for six rats in each group. All data were analyzed with SPSS/16.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. A Value of $P < 0.05$ was considered as significant.

RESULTS

Table 1 shows the qualitative analysis of phytochemicals present in the ethanolic extract of *Ananas comosus* leaves. From the preliminary phytochemical evaluation, it was found that the *Ananas comosus* leaves extract has a positive response for the presence of alkaloids, flavonoids, tannins, phytosterol, glycosides and phenols.

Table 1 Phytochemical screening of *Ananas comosus* leaves extract

PHYTOCONSTITUENTS	INFERENCE
Alkaloids	+
Flavonoids	+
Saponins	-
Tannins	+
Phytosterol	+
Diterpenes	-
Triterpenoids	-
Glycosides	+
Anthraquinones	-
Phenols	+

Table 2 shows the observed levels of body weight in control and experimental group of animals. The body weight of control rats was progressively increased whereas there was a significant decrease in the body weight of STZ induced diabetic rats. Diabetic rats treated with the leaves extract showed an improvement in body weight.

Table 2 Effect of *Ananas comosus* leaves extract on changes in body weight of experimental groups of rats after 30 days treatment.

Groups	Body weight (g)	
	Initial	Final
Control	165.24 ± 3.74	216.52 ± 5.21
Diabetic	171.25 ± 2.95	148.90 ± 7.35*
Diabetic + *Ananas comosus* leaves extract	163.19 ± 3.15	181.65 ± 5.71$^{@}$
Diabetic + gliclazide	166.14 ± 4.12	189.26 ± 6.36$^{@}$

*Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at p < 0.05. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.*

Table 3 depicts the levels of blood glucose in certain durations after the oral administration of glucose (2g/Kg body weight) in normal and experimental group of rats. In control rats, the blood glucose level reached the maximum peak at 60 min after the glucose has been loaded and then was gradually reverted back to near normal levels after 120 min.

Table 3 Effect of *Ananas comosus* leaves extract on the blood glucose level (mg/dl) in the experimental groups of rats receiving an oral glucose load

Groups	Fasting	30 min	60 min	90 min	120 min
Control	92.20 ± 6.18	150.21 ± 8.74	175.86 ± 14.57	131.39 ± 13.18	101.10 ± 9.97
Diabetic	265.89 ± 18.47*	310.63 ± 22.16*	399.84 ± 29.51*	351.17 ± 25.74*	315.83 ± 21.86*
Diabetic + *Ananas comosus* leaves extract	158.71 ± 11.54$^{@}$	188.28 ± 17.24$^{@}$	247.71 ± 22.44$^{@}$	200.07 ± 20.62$^{@}$	142.89 ± 15.70$^{@}$
Diabetic + gliclazide	142.24 ± 9.29$^{@}$	175.64 ± 15.74$^{@}$	230.20 ± 20.08$^{@}$	175.44 ± 14.71$^{@}$	130.84 ± 12.49$^{@}$

*Unit: mg/dL; Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at p < 0.05. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.*

Table 4 depicts the levels of blood glucose, glycosylated hemoglobin and urine sugar. STZ induced diabetic rats showed a significant elevation in the levels of blood glucose, presence of urine sugar and a concomitant increase in glycosylated hemoglobin. Oral administration of ethanolic extract of *Ananas comosus* leaves to the diabetic group of rats significantly reduced the levels of blood glucose and glycosylated hemoglobin. Urine sugar which was present in the diabetic group of rats was found to be absent in rats treated with the extract.

Table 4 Effect of *Ananas comosus* leaves extract on the levels of blood glucose, glycosylated hemoglobin, and urine sugar in the experimental groups of rats

Groups	Glucose (mg/dl)	Glycosylated hemoglobin (%)	Urine sugar
Control	98.09 ± 9.97	6.66 ± 1.65	Nil
Diabetic	300.08 ± 21.46*	13.18 ± 2.81*	+++
Diabetic + *Ananas comosus* leaves extract	151.20 ± 11.74$^{@}$	8.62 ± 1.71$^{@}$	Nil
Diabetic + gliclazide	126.02 ± 14.47$^{@}$	7.75 ± 2.04$^{@}$	Nil

*Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at p < 0.05. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.*

Table 5 depicts the levels of total protein, blood urea, uric acid and serum creatinine in control and experimental group of rats. In STZ induced diabetic rats, there was a significant decrease in the total protein and increase in the

levels of urea, uric acid and creatinine when compared with the control group of rats. Administration of an ethanolic extract of *Ananas comosus* leaves as well as the standard drug, gliclazide to the diabetic group of rats significantly decreased the levels of blood urea, uric acid, serum creatinine and increased the levels of total protein.

Table 5 Effect of *Ananas comosus* leaves extract on the levels of protein, urea, creatinine and uric acid in plasma of experimental groups of rats

Groups	Protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	8.02 ± 1.25	25.71 ± 2.05	1.06 ± 0.11	2.48 ± 0.92
Diabetic	5.46 ± 0.72*	46.52 ± 4.08*	2.11 ± 0.23*	5.36 ± 1.38*
Diabetic + *Ananas comosus* leaves extract	6.85 ± 0.88[@]	31.39 ± 3.22[@]	1.40 ± 0.12[@]	3.24 ± 0.98[@]
Diabetic + gliclazide	7.14 ± 0.77[@]	31.92 ± 2.88[@]	1.27 ± 0.11[@]	2.96 ± 1.12[@]

*Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [@] compared with diabetic rats.*

Table 6 depicts the levels the levels of glycogen content and activities of glycogen synthase and glycogen phosphorylase in liver tissues control and experimental groups of rats. A significant decline in the glycogen level as well as in the glycogen synthase activity and a concomitant increase in the activity of glycogen phosphorylase were noted in the liver of diabetic group of rats. Oral administration of *Ananas comosus* leaves extract as well gliclazide to diabetic rats restored the level of glycogen and the activities of glycogen synthase, glycogen phosphorylase to near normalcy when compared to control group of rats.

Table 6 Effect of *Ananas comosus* leaves extract on the levels of liver glycogen content in the experimental groups of rats

Groups	Glycogen	Glycogen synthase	Glycogen phosporylase
Control	40.56 ± 3.52	804.18 ± 42.72	616.76 ± 32.60
Diabetic	18.64 ± 2.18*	520.30 ± 27.35 *	862.71 ± 49.09 *
Diabetic + *Ananas comosus* leaves extract	32.81 ± 3.76[@]	719.46 ± 30.07 [@]	670.18 ± 30.38 [@]
Diabetic + gliclazide	30.78 ± 2.95[@]	734.40 ± 49.34 [@]	690.75 ± 32.20[@]

*Units are expressed as: mg/g wet tissue for glycogen, µmoles of UDP formed/h/mg protein for glycogen synthase and µ moles of Pi liberated/h/mg protein for glycogen phosphorylase. Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [@] compared with diabetic rats.*

Table 7 depicts the levels of aspartate transaminase, alanine transaminase and alkaline phosphatase in the control and experimental group of rats. Diabetic rats showed a significant elevation in the levels of aspartate transaminase, alanine transaminase and alkaline phosphatase when compared with the control group of rats. Administration of *Ananas comosus* leaves extract and gliclazide to the diabetic rats resulted in a significant decrease in the levels of these markers.

Table 7 Effect of *Ananas comosus* extract on the activity of AST, ALT and ALP in the serum of experimental groups of rats

Groups	AST	ALT	ALP
Control	59.75 ± 7.21	16.39 ± 3.14	76.84 ± 9.78
Diabetic	108.27 ± 15.36*	52.26 ± 7.51*	185.27 ± 18.41*
Diabetic + *Ananas comosus* leaves extract	72.52 ± 12.41[@]	28.37 ± 3.82[@]	95.61 ± 10.84[@]
Diabetic + gliclazide	79.15 ± 9.54[@]	22.48 ± 4.16[@]	109.75 ± 12.97[@]

*The enzyme activities are expressed as: AST and ALT µmoles of pyruvate liberated /h/mg of protein; ALP µmoles of phenol liberated/min/mg of protein. Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [@] compared with diabetic rats.*

Table 8 Effect of *Ananas comosus* leaves extract on the level of TBARS in plasma and pancreas, of experimental groups of rats

Groups	TBARS	
	Plasma	Pancreas
Control	3.94 ± 0.83	35.49 ± 5.26
Diabetic	7.62 ± 1.90*	70.57 ± 12.37*
Diabetic + *Ananas comosus* leaves extract	4.91 ± 1.68[@]	44.78 ± 10.64[@]
Diabetic + gliclazide	5.26 ± 1.59[@]	53.85 ± 12.35[@]

*Units: mM/100 g in tissues; nM/ml in plasma. Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [@] compared with diabetic rats.*

The levels of TBARS in the plasma and pancreas of control and experimental group of rats are presented in **Table 8**. STZ induced diabetic rats showed marked increase in the levels of TBARS when compared to control rats.

Treatment of *Ananas comosus* leaves extract as well as Gliclazide to the diabetic rats showed a significant decrease in the levels of these oxidative stress markers.

Table 9 and 10 illustrates the activities of enzymatic and non enzymatic antioxidants in plasma of control and experimental group of rats. In STZ induced diabetic rats, there was a significant reduction in the activities of enzymatic and non enzymatic antioxidants in plasma when compared to the control rats. Treatment of *Ananas comosus* leaves extract to the diabetic rats showed improvement in the activities of enzymatic and non enzymatic antioxidants.

Table 9 Effect of *Ananas comosus* leaves extract on the activity of SOD, Catalase and GPx, and the level of GSH in pancreas of experimental groups of rats.

Groups	SOD	Catalase	GPx	GSH
Control	4.29 ± 1.45	13.79 ± 2.72	6.68 ± 1.59	22.37 ± 3.42
Diabetic	1.92 ± 0.95*	6.48 ± 1.96*	3.45 ± 0.99*	10.96 ± 2.71*
Diabetic + *Ananas comosus* leaves extract	3.74 ± 1.27$^{@}$	10.14 ± 2.19$^{@}$	4.82 ± 1.16$^{@}$	16.94 ± 2.99$^{@}$
Diabetic + gliclazide	3.68 ± 0.98$^{@}$	9.64 ± 1.93$^{@}$	4.91 ± 1.25$^{@}$	18.37 ± 3.14$^{@}$

*Activity is expressed as: 50% of inhibition of epinephrine autooxidation/min/mg of protein for SOD; µmoles of hydrogen peroxide decomposed/min/mg of protein for catalase; µmoles of glutathione oxidized/min/mg of protein for GPx; mg/100 g tissue for GSH. Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at p < 0.05. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.*

Table 10 Effect of *Ananas comosus* leaves extract on the levels of vitamin C, vitamin E, ceruloplasmin and GSH in plasma of experimental groups of rats

Groups	Vitamin C	Vitamin E	Ceruloplasmin	GSH
Control	1.57 ± 0.28	0.72 ± 0.14	10.96 ± 2.61	25.94 ± 4.53
Diabetic	0.52 ± 0.12*	0.38 ± 0.09*	4.99 ± 1.24*	12.76 ± 2.71*
Diabetic + *Ananas comosus* leaves extract	1.15 ± 0.15$^{@}$	0.61 ± 0.10$^{@}$	7.82 ± 1.72$^{@}$	18.56 ± 3.69$^{@}$
Diabetic + gliclazide	1.20 ± 0.12$^{@}$	0.56 ± 0.09$^{@}$	8.55 ± 2.17$^{@}$	16.97 ± 2.84$^{@}$

*Units: mg/dl. Values are given as mean ±SD for groups of six rats in each. Values are statistically significant at p < 0.05. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.*

DISCUSSION

Streptozotocin is a nitrosourea analogue, preferentially uptaken by pancreatic beta cells via GLUT2 glucose transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD^{+} and ATP. Enhanced ATP dephosporylation after STZ treatment provides substrate for xanthine oxidase resulting in the formation of superoxide radicals. Further, NO moiety is liberated from STZ leading to the destruction of β cells by necrosis. Hence STZ-induced experimental diabetes is chosen as the animal for the present study [41].

Phytochemicals are bioactive compounds (secondary metabolites) found in plants that works synergistically with nutrients and dietary fibers to protect against diseases[42]. Most plants with antidiabetic properties have been found to contain secondary metabolites such as glycosides, alkaloids and flavonoids [43]. It has been shown that many plants exhibit efficient antioxidant properties owing to their phenolic constituents. *Ananas comosus* leaves extract has a positive response for the presence of Alkaloids, Flavonoids, Tannins, Phytosterol, Glycosides and Phenols.

Diabetes mellitus results in loss of body weight [44, 45]. The body weight of control rats was progressively increased whereas there was a significant decrease in the body weight of STZ induced diabetic rats. Decrease in the body weight due to derangement of metabolic pathways is a common feature in diabetes [46] which might be due to the breakdown of tissue proteins. Diabetic rats treated with *Ananas comosus* leaves extract and gliclazide for 30 days showed a significant improvement in body weight indicating the beneficial effect of the leaves extract in controlling muscle wasting.

Oral Glucose Tolerance Test (OGTT) measures the body's ability to utilize glucose, the body's main source of energy. An oral glucose tolerance test is a more sensitive measure of early abnormalities in glucose regulation than fasting plasma glucose [47]. The glucose tolerance test was devised in order to measure the metabolic response of a patient to oral glucose. In STZ induced diabetic rats, the blood glucose level increased to peak at 60 min and remained high over the next 60 min. *Ananas comosus* leaves extract and the gliclazide treated diabetic rats showed an increase at 60 min and then the reduction in peak was observed at 120 min which finally exhibited the near normal range of control rats. The results obtained from GTT indicate the improved glucose utilization in diabetic rats treated with the leaves extract.

Blood glucose is an index for the diagnosis of diabetes mellitus. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus [48]. In the present study, diabetic rats showed elevation in blood glucose level which upon oral administration with *Ananas comosus* leaves extract resulted in a significant reduction of blood glucose levels indicating the antihyperglycemic nature of the leaves extract.

Persistent hyperglycemia leads to the glycosylation of amino groups of lysine residue in proteins. Non-enzymatic glycosylation of protein occurs by direct reaction between reducing sugars and certain amino groups in protein. This condition favors reduction in the level of total hemoglobin and elevation in glycosylated hemoglobin, which is directly proportional to persistant blood glucose levels [49]. Diabetic rats showed higher levels of glycated hemoglobin indicating their poor glycemic control. Oral administration of the leaves extract to diabetic rats decreased the level of glycosylated hemoglobin indicating the improved glucose homeostasis. Urine sugar which was present in diabetic groups of rats were absent in the rats treated with the leaves extract indicating the improved glycemic status. It has been previously reported that *Ananas comosus* improve insulin sensitivity in type 2 diabetic rats [15].

The level of total proteins is found to decrease in diabetic group of rats. The deficiency of insulin leads to defective amino acid/protein metabolism, which may be a more important factor than hyperglycemia in the etiology of some diabetic complications [50]. In the present study, administration of *leaves* extract to the diabetic rats significantly inhibits proteolysis caused by insulin deficiency and thus increased the levels of total proteins.

Impaired balance of nitrogen coupled with lowered protein synthesis leads to the increased concentration of urea in blood [51]. Elevated levels of creatinine and urea in the serum are well documented as one of the most sensitive markers of kidney damage. Uric acid is the main catabolic product from purine nucleotides by xanthine oxidase enzymatic system. It is considered as a biomarker for the development of diabetic complications. Serum uric acid has been shown to be associated with oxidative stress and the production of tumour necrosis factor-α [52] in which both are directly related to the development of diabetes. The levels of urea, uric acid and creatinine was found to be increased significantly in STZ-induced diabetic rats when compared to control rats. The observed increase might be due to the STZ-induced metabolic disturbances. In the present study, administration of *Ananas comosus* leaves extract to the diabetic rats has decreased the levels of urea, uric acid and creatinine to near normal range. The observed effects of *Ananas comosus* was equivalent to that of the standard drug, gliclazide.

Glycogen is the primary intracellular storage form of glucose and its levels in various tissues are a direct reflection of insulin activity because insulin promotes glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase activities [53]. Evidence shows that the diabetes mellitus is associated with a marked decrease in the levels of liver glycogen [54]. Glycogen synthase is a crucial and rate-limiting enzyme which catalyzes the transfer of glucose from UDP-glucose to glycogen. Glycogen phosphorylase is a rate-limiting enzyme of glycogenolysis and is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose-6-phosphate and glucose [55]. During diabetic conditions, the glycogen levels, glycogen synthase activity and responsiveness to insulin signaling are diminished and glycogen phosphorylase activity is significantly increased. Oral administration of leaves to diabetic rats restored the glycogen content and the activities of glycogen metabolizing enzymes demonstrating the possible role of leaves in the regulation of glycogen metabolism.

Aspartate transaminase (AST) and Alanine transaminase (ALT) are the enzymes which are associated with the conversion of aminoacids to ketoacids and are used as marker enzymes to assess tissue damage [56]. The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissues and migrating into the blood stream. The increase in serum alkaline phosphatase activity that were formed in liver and released into bile is a sign of a loss of the secretory function of the liver [57]. The observed increase in activities of these enzymes in the serum of diabetic rats may be due to the leakage of these enzymes from the liver cytosol into blood stream as a consequence of the hepatic tissue damage. The activities of these marker enzymes was found to be normalized upon treatment with the leaves extract indicating the non toxic as well as hepatoprotective nature of the *Ananas comosus* leaves.

Lipid peroxides and hydroperoxides are the secondary products of oxidative stress and are unleashed as a result of the toxic effect of reactive oxygen species produced during lipid peroxidation in diabetes [58]. Induction of diabetes in rats with STZ uniformly results in an increase in thiobarbituric acid reactive substances (TBARS), an indirect evidence of free-radical production [59]. The elevated levels of the oxidative stress marker in diabetic rats were reduced significantly upon treatment with the leaves extract indicating its anti-lipid peroxidative nature.

Persistent hyperglycemia causes increased oxidative stress, which contributes to the development and progression of most of the diabetes-associated complications. Having evolved in an oxygen environment, most cells, including pancreatic beta-cells, have acquired intricate mechanisms to defend against ROS toxicity. However, the reduced antioxidant capacity potentially makes pancreatic β-cells sensitive to ROS mediated signal transduction and cellular response. Thus, maintenance of β-cell oxidant status and their protection against oxidative damage might delay the onset of diabetes as well as the progression of its complications.

SOD is an important defense enzyme, scavenges O_2^- anion to form H_2O_2 and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reaction. Catalase activity is largely located in subcellular organelles known as peroxisomes. CAT is a hemoprotein which catalyzes the reduction of hydrogen peroxides and known to be involved in detoxification of H_2O_2 concentrations. Glutathione peroxidase (GPx) is a selenoprotein, first described as an enzyme that protects hemoglobin from oxidative degradation in red blood cells. Oxidative stress in diabetes coexists with reduction in antioxidant capacity, which can increase the deleterious effects of free radicals and consequently leads to long-term complications of diabetes [60]. The decreased enzymatic antioxidant status observed in diabetic rats was improved upon treatment with the leaves extract indicating the free radical scavenging activity of the leaves extract.

In addition to enzymatic antioxidants, the endogenous non-enzymatic antioxidants GSH and ceruloplasmin and the dietary antioxidants vitamin C and vitamin E play a significant role in the maintenance of antioxidant status. In the diabetic condition, because of hyperglycemia-mediated increases in oxidative stress, these antioxidants are depleted [61 - 64]. In the present study, oral administration of the leaves extract significantly improved levels of vitamin C, vitamin E, ceruloplasmin, and GSH, which may be due to the sparing action of the leaves extract in the scavenging of radicals. *Ananas comosus* leaves possess hypoglycemic and anti-oxidative activities in diabetic rats [15].

CONCLUSION

The results of the present study indicate that *Ananas comosus* leaves extract possess significant antidiabetic as well as antioxidant properties. Phytochemical screening analysis indicated the presence of biologically active ingredients in the leaves. The results of the OGTT indicate the improved glucose homeostasis upon the treatment. The improvement in bodyweight indicates the beneficial effect of the extract. The detailed observation of the biochemical analysis has revealed that the plant extract significantly alters the biochemical parameters in plasma, serum and certain vital tissues. The normalization in the activities of pathophysiological enzymes indicates the non toxic nature of the extract. The improved antioxidant status indicates the antioxidant property of the leaves extract. In conclusion, the synergistic secondary metabolites present in the extract may account for the antidiabetic property of the leaves. Further studies are in progress to isolate and identify the phytochemicals responsible for the observed beneficial as well as pharmacological activities of *Ananas comosus* leaves.

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