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Effect of *Psidium guajava* leaf extract, glibenclamide and their combination on rat model of diabetes induced by streptozotocin

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ABSTRACT

Aim: The present aim is to evaluate the anti-diabetic effect of aqueous extract of guava (*Psidium guajava*) leaf using rat model of diabetes induced by streptozotocin. In addition, the effect of this extract on liver and kidney functions induced in rat model of diabetes were investigated.

Material and Methods: Rats were divided randomly into: control group, rat model of diabetes induced by streptozotocin, rat model of diabetes treated with aqueous extract of guava leaf, rat model of diabetes treated with glibenclamide and rat model of diabetes treated with aqueous extract of guava leaf plus glibenclamide.

Result: In the present rat model of diabetes a significant decrease in the serum insulin level and a significant increase in glucose level were detected. Streptozotocin induced a significant increase in the activities of AST, ALT, ALP and a significant increase in the levels of bilirubin, urea, creatinine and uric acid. In addition histopathological and immunological changes were detected in the pancreatic tissue. The present data revealed that aqueous extract of guava leaf improved the reduced insulin level and the high glucose level induced by streptozotocin. This was associated with an improvement in the changes in the liver and kidney functions. Loss of body weight gain induced by streptozotocin was alleviated by guava leaf extract, glibenclamide or both.

Conclusion: According to the present findings it could be concluded that the aqueous extract of guava leaf has a potent anti-hyperglycemic effect on rat model of diabetes induced by streptozotocin with hepatic and renal protective effects.

Key words: Hypoglycemic, Streptozotocin, *Psidium guajava*, liver functions, kidney functions, Rats

INTRODUCTION

Diabetes mellitus (DM) is a disease characterized by high blood sugar (glucose) levels that result from defects in insulin secretion, insulin action, or both. The chronic metabolic imbalance associated with this disease puts patients at high risk for long-term macro- and micro-vascular complications. These vascular changes may lead to development of clinical complications characteristically affecting the eye, the kidney and the nervous system [(1)].

It has been estimated that world-wide prevalence of diabetes mellitus in 2008 was more than 347 million with varying prevalence among different ethnic groups, and it is expected to rise to 500 million by 2025 [(2)].

Complications of diabetes typically take five to ten years to manifest themselves. They are generally irreversible and are predominantly related to sustained high levels of blood glucose. These complications lead eventually to death or disability and can be mostly avoided by adequate glycemic control**.** Tissue damage associating diabetes occurs through five major mechanisms: increased flux of glucose and other sugars through the polyol pathway; increased intracellular formation of advanced glycation end products (AGEs); increased expression of the receptor for AGEs and its activating ligands; activation of protein kinase C isoforms; and over activity of the hexosamine pathway. All these five mechanisms are activated by a single upstream event which is mitochondrial overproduction of reactive oxygen species [(3)].

Oxidative stress plays a key role in the pathogenesis of micro- and macro-vascular diabetic complications. The increased oxidative stress in diabetic patients is a consequence of several abnormalities including hyperglycemia and insulin resistance[(4)].

Diabetes mellitus is a chronic disease that can be controlled by life styles such as monitoring ones weight, diet, exercise to long term use of oral hypoglycemia drugs. Treatment can also be achieved by the use of synthetic drugs such as sulphonylureas**.** Herbs and vegetables have contributed significantly in improvement of human health in terms of prevention and/or treatment of diseases. Thousands of wild plant species grow in Africa and have both nutritional and therapeutic purposes [(5)].

Psidium guajava, is an important food crop and medicinal plant in tropical and subtropical countries which is widely used in medicine around of the world. *P. guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhea and dysentery [(6)]. It has also been used extensively as a hypoglycemic agent.

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Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepato-protective, antiallergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses [(7)].

Due to the side effects that have been reported with antidiabetic agent such as hypoglycemia, headache, dizziness, nausea, hypersensitivity reactions, and weight gain, hepatic and renal diseases [(8)], there is a persistent needs to search safe and effective alternatives.

Therefore, the present study was conducted to investigate the hypoglycemic effect of aqueous extract of guava leaf on rat model of diabetes induced by streptozotocin solely or in combination with glibenclamide. This was achieved by determining the effects of guava leaf extract on the changes in the levels of insulin and glucose and on the histopathological and immunological changes in pancreatic tissue induced by streptozotocin. The study was extended to evaluate the effects of guava leaf extract on the changes in body weight gain, liver and kidney functions induced in rat model of diabetes. The efficacy of extract of guava leaf as antidiabetic agent will be evaluated by comparing its findings with those of the well-established antidiabetic agent glibenclamide.

MATERIALS AND METHODS

Experimental animals

Adult male Wistar albino rats weighing 120-150 g were obtained from Schistosoma Biological Supply Program (**SBSP**), Theodor Bilharz Research Institute, Cairo, Egypt. During the study period, each two rats were placed in a metal cage. They were kept under normal laboratory conditions during the whole period of the experiment. All rats were fed on standard food and they were left for free access to water and food, with 12 hour day and night cycle. They were kept for 10 days for the adaptation before the start of the experiment.

Medicinal plants and chemical compounds

Streptozotocin

Streptozotocin (STZ) 2-Deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose, (Batch No.126k1174) was purchased from Sigma-Aldrich (Germany) and was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 60 mg/kg b.w. and injected intraperitoneally within 15 minutes of dissolution to obtain rat model of diabetes.

Glibenclamide

Glibenclamide is an antidiabetic drug that belongs to a class of medications known as sulfonylureas. Glibenclamide,5 Chloro N (4[N (cyclohexylcarbamoyl) sulfamoyl] phenethyl) -2-methoxy benzamide, was purchased from Hoechst Pharm. Co. (Germany). The tablets were crushed, suspended in distilled water and given to diabetic rats at the dose level 5 mg/kg body weight daily by gastric intubation.

Psidium guajava leaf extract (guava leaf extract)

Psidium guajava belongs to family Myrtaceae. Fresh leaves of ***P. guajava*** were collected from a farm at Al-Sharkia, Egypt. The leaves were sorted to eliminate any dead matter and other unwanted particles. The leaves were air-dried for 2 weeks and then ground into fine powder using an electric dry mill. A total of 200 g of the ground powder was soaked in one liter of distilled water for 48 hours at room temperature. The mixture was filtered into 600 ml conical flask with filter paper. The filtrate was dried at a temperature of 30 °C for 6 hours to produce a 200 ml gel-like extract. The appropriate concentration of the extract was diluted with distilled water to obtain the used oral dose of guava leaf extract (500 mg/kg body weight).

Induction of diabetes

The rat model of diabetes was induced by a single intra-peritoneal (i.p.) injection of STZ at a dose of 60 mg/ kg body weight diluted in 0.4 ml of freshly prepared citrate buffer (pH 4.5) after an overnight fast**.** After 48 hours of SZT injection, the development of rat model of diabetes was confirmed by measurement of tail vein blood glucose level using Glucotrend 2 glucometer (One Touch Technology, Roche Group, UK). Rats with blood glucose levels above 350 mg/dl were considered diabetic and were included in the study. Treatment was started after 48 hours of induction of diabetes and continued for 4 weeks. Fasting blood glucose was then measured in the tail vein blood for all groups after 2, 15 and 30 days.

Experimental design

At the start of the study, the rats were divided randomly into two groups: **the first group** was the control group (n=6): rats received a single i.p. physiological saline (0.5 ml/rat) followed by daily oral physiological saline solution (0.9%) 0.5

ml / rat for 4 weeks and represented the negative control. **The second group** was the rat model of diabetes (n=24): rats received a single i.p. dose of SZT (60 mg/kg) to obtain the rat model of diabetes. The diabetic rats were subdivided into four subgroups (6 each): **Subgroup-1**: Rat model of diabetes, the positive control that didn't receive any medication. **Subgroup-2**: Rat model of diabetes treated daily with guava leaf extract (500 mg/ kg, orally) for 4 weeks. **Subgroup-3**: Rat model of diabetes treated daily with glibenclamide drug at a dose of 5 mg/kg orally for 4 weeks. **Subgroup-4:** Rat model of diabetes received a combined oral treatment of daily guava leaf extract (500 mg/kg) and glibenclamide (5 mg/kg) for 4 weeks.

At the end of the experiment rats were fasted overnight, the rats were anaesthetized by placing them in an anesthetic box filled with ether vapor maintained by periodically applying liquid to a cotton wool on the base of the box. Then the animals (both control and treated ones) were sacrificed and blood samples were collected for biochemical studies.

Determination of Body weight gain

The body weight was measured daily for each rat then the body weight gain was estimated by subtracting the initial body weight from the final body weight for each rat.

Biochemical analyses

Blood Samples were collected and left to clot in a clean dry centrifuge tube for each rat, then centrifuged at 5000 r.p.m. for 15 minutes. A portion of the clear supernatant serum was used immediately for glucose determination. The remaining serum was frozen at –20ºC for subsequent analysis.

Determination of serum glucose level

Serum glucose level was measured according to the enzymatic colorimetric method described by Trinder**.**

Determination of serum insulin level

Serum insulin concentration was measured by 1-step chemiluminescence sandwich (immunoassay (CLIA) method (LIAISON analyzer (310360) Diasorin S.P.A, Vercelli, Italy)**.**

Determination of Liver functions

Liver functions were determined by measuring the activities of the following enzymes in the serum: Aspartate aminotransferase (AST) activity was estimated according to the kinetic method of Tietz. Alanine aminotransferase (ALT) activity was estimated according to International Federation of Clinical Chemistry. Alkaline phosphatase (ALP) activity was estimated according to Englehardt. In addition the total bilirubin was determined in the serum according to the method described by Tietz.

Determination of Kidney Functions

Kidney functions were determined by measuring serum urea level**,** serum uric acid level using the method described by Young and serum creatinine level as described by Bartels and Bohmer method.

Statistical Analysis

The obtained data were statistically analyzed using SPSS program according to the method of Glantz. Significant differences among groups were determined by one-way analysis of variance (ANOVA). This was followed by post hoc test using Duncan test to compare significance between groups at p-value < 0.05.

RESULTS

Body weight gain results

As shown in table (1) a significant decrease in the body weight gain was observed in diabetic rats recording -88 % lower than the control values. Body weight gain decreased significantly by -21.7% in diabetic rats treated with guava leaf and increased significantly by 11.1% in diabetic rats treated with glibenclamide as compared to control value. The body weight of the diabetic rats treated with guava leaf extract and glibenclamide showed a significant decrease (-30.3%). ANOVA revealed also that the average weight gain of the animals treated with extract, drug or combination increased significantly as compared to untreated diabetic control group.

Biochemical results

As illustrated in table (2) serum glucose level increased significantly in rats treated with streptozotocin recoding 228.9 % and 226.9% after 15 and 30 days respectively as compared to control. When the animal model of diabetes was treated daily with guava leaf extract for 15 and 30 days, the elevated glucose level induced by STZ decreased to 61.5% and 15.7% as compared to control values, respectively. The daily treatment with glibenclamide for 15 and 30 days reduced the glucose level of the diabetic rats to 100% and 42.3% respectively as compared to control values.

Glucose level in rat model of diabetes co-treated daily with guava extract and glibenclamide decreased to 71% after 15 days and was restored to control like value (2.1%) after 30 days. It is clear from the present data that in diabetic rats treated with guava leaf extract, glibenclamide and their combination the glucose level decreased significantly as compared to untreated diabetic rats.

ANOVA revealed that after 30 days of single STZ injection a significant decrease (-67.4%) in serum insulin level was observed in comparison to control value. Although the daily oral administration of the diabetic rat with guava leaf extract for 30 days improved the level of insulin to -15.3%, it continued to show a significant decrease as compared to the control rats and a significant increase as compared to diabetic rats. Insulin level decreased significantly by -34.7% in the diabetic rats treated with glibenclamide only as compared to control rats. When the diabetic rats were co-treated with both guava leaf extract and glibenclamide, insulin level decreased significantly recording – 16.4 % below the control value (table 3).

The effect of guava leaf extract, glibenclamide and their combined administration on the liver function was illustrated in table (4). The present data revealed significant increases in the activities of AST, ALT and ALP in the serum of streptozotocin-induced diabetic model recording 107%, 253.1% and 176.8% above the control values, respectively. This was accompanied by a significant increase in serum bilirubin level (465.9%). The daily treatment of rat model of diabetes with guava leaf extract restored the activities of AST, ALT and ALP and the bilirubin level to control like values (-1.9%, 5.1%, 2.6% and 5.4% respectively).

However, the daily treatment of diabetic rats with glibenclamide for 30 days resulted in a significant increase in the activities of sAST, sALT, sALP and bilirubin level recording 130%, 27.8%, 104.3% and 353.5% respectively as compared to control values. It is clear from the present findings that glibenclamide failed to restore the increased activities of AST, ALT and ALP and the elevated level of bilirubin induced in rat model of diabetics to control values. In the rat model of diabetes co-treated with guava leaf extract and glibenclamide, control like values of sAST (6.4%) and sALP (-2.3%) were observed. Although the co-treatments decreased the elevated activity of sALT (23.2%) and the elevated level of bilirubin (35.6%) induced by streptozotocin, their values still showed significant increases above the control values.

The data showing the effect of guava leaf extract, glibenclamide and their combined administration on the kidney functions were presented in table (5). In the rat model of diabetes, a significant increase in the serum level of creatinine (45.2%), urea (53.9%) and uric acid (29.5%) was obtained at comparing the data with the control values. Creatinine level was improved to 17.9% after the daily treatment with guava leaf extract. Moreover, guava leaf extract ameliorated the increased urea level (-3.4%) and reversed the significant increase in uric acid induced by streptozotocin to a significant decrease (-25.9%). The daily treatment of diabetic model with glibenclamide normalized the creatinine level (0.0% difference from control), improved the significant increase in urea (36.6%) and reversed the significant increase in uric acid to a significant decrease (-28.5%). When the rat model of diabetes was co-treated daily with guava leaf extract and glibenclamide, a significant increase in serum creatinine level (22.6%) was recorded, that was accompanied by a significant decrease in urea level (-14.9%) and a control like value of uric acid (-6.2%) in comparison to control values.

Table (1): The effect of daily oral administration of *Psidium Guajava* leaf extract (GU) (500 mg/kg b.w), glibenclamide (GLB) (5 mg/kg b.w) and GU+GLB for 30 days on the change in body weight gain (gm) induced in rat model of diabetes.

Groups	Control	Diabetic rats	% D	Diabetic rats treated with Gu	%D	Diabetic rats treated with GLB	%D	Diabetic rats treated with GU+GLB	%D	p-value
b.w change	77.66^{a}±1.74 (6)	9.33^{b}± 2.34 (6)	-88%	60.83^{c} ± 3.01 (6)	21.7%	86.33^{d}± 2.85 (6)	11.1%	54.16^{c} ± 3.91 (6)	30.3%	<0.0001

Values represent mean ± S.E.
Number of rats was 6 rats per group
% D: % difference with respect to control values [(Treated value - Control value)/Control value] x 100
Different letters indicate significantly different means p-value < 0.05
Same letters indicate non-significant changes

Table (2): The effect of daily oral administration of *Psidium Guajava* leaf extract (GU) (500 mg/kg b.w), glibenclamide (GLB) (5 mg/kg b.w) and GU+GLB for 15 and 30 days on the serum level of glucose (md/dl) in rat model of diabetes induced by streptozotocin.

Groups Time	Control	Diabetic rats	% D	Diabetic rats treated with Gu	%D	Diabetic rats treated with GLB	%D	Diabetic rats treated with GU+GLB	%D	p-value
15 days	108.33a±1.706 (6)	356.33b±1.763 (6)	228.9%	175.00c±1.861 (6)	61.5%	216.67d±1.626 (6)	100%	185.33e±1.819 (6)	71%	<0.001
30 days	110.00a±1.390 (6)	359.67b±2.170 (6)	226.9%	127.33c±2.027 (6)	15.7%	156.50d±1.477 (6)	42.3%	112.33a±1.429 (6)	2.1%	<0.001

Values represent mean ± S.E. with the number of animals between parenthes
% D: % difference with respect to control values [(Treated value - Control value)/Control value] x 100
Different letters indicate significantly different means p-value < 0.05
Same letters indicate non-significant changes.

Table (3): The effect of daily oral administration of *Psidium Guajava* leaf extract (GU) (500 mg/kg b.w), glibenclamide (GLB) (5 mg/kg b.w) and GU+GLB for 30 days on the serum level of insulin (ng/l) in rat model of diabetes induced by streptozotocin.

Groups parameter	Control	Diabetic rats	% D	Diabetic rats treated with Gu	%D	Diabetic rats treated with GLB	%D	Diabetic rats treated with GU+GLB	%D	p-value
Insulin	16.33a ± 0.210 (6)	5.33b ± 0.210 (6)	-67.4%	13..83c ± 0.307 (6)	-15.3%	10.66d ± 0.333 (6)	-34.7%	13.66c ± 0.494 (6)	-16.4%	<0.001

Values represent mean ± S.E. with the number of animals between parentheses
% D: % difference with respect to control values [(Treated value - Control value)/Control value] x 100
Different letters indicate significantly different means p-value < 0.05
Same letters indicate non-significant changes.

Table (4): The effect of daily oral administration of *Psidium Guajava* leaf extract (GU) (500 mg/kg b.w), glibenclamide (GLB)(5 mg/kg b.w) and GU+GLB for 30 days on the activity of AST, ALT, ALP and the level of Bilirubin (mg/dl) of rat model of diabetes.

Groups parameter	Control	Diabetic rats	% D	Diabetic rats treated with Gu	%D	Diabetic rats treated with GLB	%D	Diabetic rats treated with GU+GLB	%D	p-value
AST	161.50a±1.118 (6)	334.33b±1.909 (6)	107%	158.33a ±1.726 (6)	-1.9%	371.50c ±1.500 (6)	130%	171.83d ± 1.515 (6)	6.4%	<0.001
ALT	64.67a ± 0.843 (6)	228.33b±1.382 (6)	253.1%	68.00a ± 1.000 (6)	5.1%	82.67c ±2.092 (6)	27.8%	79.67c ± 1.229 (6)	23.2%	<0.001
ALP	252.67a±2.458 (6)	699.33b±2.124 (6)	176.8%	259.17a ±2.574 (6)	2.6%	516.33c ±2.431 (6)	104.3%	246.83a ± 2.903 (6)	-2.3%	<0.001
Bilirubin	0.258a ± 0.003 (6)	1.46b ± 0.042 (6)	465.9%	0.272a ± 0.003 (6)	5.4%	1.17c ± 0.003 (6)	353.5%	0.350d ± 0.019 (6)	35.6%	<0.001

Values represent mean ± S.E. with the number of animals between parentheses
% D: % difference with respect to control values [(Treated value - Control value)/Control value] x 100
Different letters indicate significantly different means p-value < 0.05
Same letters indicate non-significant changes

Table (5): The effect of daily oral administration of *Psidium Guajava* leaf extract (GU) (500 mg/kg b.w), glibenclamide (GLB)(5 mg/kg b.w) and GU+GLB for 30 days on level of creatinine, urea and uric acid of rat model of diabetes induced by streptozotocin.

Groups / parameter	Control	Diabetic rats	% D	Diabetic rats treated with Gu	%D	Diabetic rats treated with GLB	%D	Diabetic rats treated with GU+GLB	%D	p-value
Creatinine	0.367a ± 0.021 (6)	0.533b ± 0.021 (6)	45.2%	0.433c ± 0.021 (6)	17.9%	0.367 a± 0.021 (6)	0.0%	0.450c ± 0.022 (6)	22.6%	<0.001
Urea	67.58a ± 1.124 (6)	104.00b±1.154 (6)	53.9%	65.28a ± 1.231 (6)	-3.4%	92.35c ± 1.742 (6)	36.6%	57.53d ± 1.106 (6)	-14.9%	<0.001
Uric acid	1.12a ± 0.040 (6)	1.45b ± 0.088 (6)	29.5%	0.83c ± 0.021 (6)	-25.9%	0.80 c± 0.036 (6)	-28.5%	1.05a ± 0.080 (6)	-6.2%	.000

Values represent mean ± S.E. with the number of animals between parentheses
% D: % difference with respect to control values [(Treated value - Control value)/Control value] x 100
Different letters indicate significantly different means p-value < 0.05
Same letters indicate non-significant changes.

DISCUSSION

In the present study, the anti-diabetic effect of guava leaf extract was investigated using rat model of type-1 diabetes induced by streptozotocin (STZ). Streptozotocin (50-100 mg/kg B.W) induced rat model of diabetes is probably the most commonly used experimental model of type-1 diabetes in which diabetes develops within 1-2 days**.**The present findings indicate that SZT injection resulted in a significant decrease in serum insulin level as compared to control value. This was associated with a significant increase in serum glucose level. The same results were obtained by [(9)]**.**

Due to its similarity with glucose, streptozotocin can bind with glucose transporter receptor (GLUT2) and accumulates in the pancreatic β cells. Therefore, STZ targets β cells by its alkylating property corresponding to that of cytotoxic nitrosourea compounds**.** STZ is a potent DNA methylating agent, acting as nitric oxide donor in pancreatic cells. β-Cells are particularly sensitive to damage by nitric oxide and free radicals because of their low level of free radicals scavenging enzymes, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues. Under this condition, the destruction of β cells and induction of the hyperglycemic state are associated with inflammatory infiltrates including lymphocytes in the pancreatic islets [(10)]**.**

Therefore, the present decrease in serum insulin level could be attributed to the destruction of pancreatic β cells, resulting in hypoinsulinemia. As a result of this effect, SZT induced hyperglycemia that was recorded in the present study after 15 and 30 days of a single dose of SZT. Moreover, the persistency of the elevated level of serum glucose after 15 and 30 days indicates the stability and establishment of rat model of type-1 diabetes.

Streptozotocin prevents DNA synthesis in mammalian and bacterial cells. In bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The biochemical mechanism results in mammalian cell death. Streptozotocin prevents cellular reproduction with a much smaller dose than the dose needed for inhibiting the substrate connection to the DNA or inhibiting many of the enzymes involved in DNA synthesis [(11)]. Thus the stability of this model could be attributed to the irreversible destruction of Langerhans islets cells.

The lowering of glucose levels by aqueous extract of *P. guajava* could be attributed to the high content of quercetin in *P. guajava* leaf extract thereby confirming its usefulness for diabetic patients. Quercetin in aqueous extract of *P. guajava* leaf promotes glucose uptake in liver cells which contributes to the control of hyperglycemia in diabetes**.** Guava leaf extracts could inhibit protein tyrosine phosphatase 1B activity and alpha-glucosidase enzymes to regulate glucose metabolism in db/db mice. Additionally, it could promote glucose uptake in hepatocytes and increase the activities of hepatic hexokinase and glucose-6-phosphate dehydrogenase in the liver of STZ-induced diabetic rats [(12)]**.** Also, **Rawi *et al*.**[(12)] suggested that the anti-diabetic activity of the *P. guajava* may be possible through various mechanisms such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous

glucose uptake by peripheral tissue, or activation of gluconeogenesis in liver and muscles.

Furthermore, **Wang *et al.*** [13] found that the aqueous extract from guava leaves inhibited both sucrase and maltase activities in the small intestinal mucosa of diabetic mice, occurring as a mixed type of competitive and non-competitive inhibition. Several enzymes secreted by the small intestine, namely maltase, lactase, sucrase, and lipase, are generally known to break down those polysaccharides and lipids into monosaccharides and free fatty acids. Disaccharides and lipids are then converted into monosaccharides by disaccharidases located in the small intestinal mucosa**.** The inhibition of the activity of these enzymes in the human digestive tract is considered to be effective in the control of diabetes through diminishing the absorption of glucose that these enzymes break down from starch. Moreover, if α-glucosidases are inhibited, the liberation of D-glucose from dietary complex carbohydrates can be retarded. Alpha-glucosidase inhibitors have been known as one of therapeutic approaches for diabetes mellitus since the early 1990s**.** The study of **Wang *et al.*** [14] reported that guava leaf extract has an inhibitory effect on α-glucosidase and α-amylase.

Therefore, the present recorded decrease in serum glucose level induced by daily treatment with guava leaf extract could be mediated by two mechanisms. The first mechanism involves promoting glucose uptake in hepatocytes and increasing the activities of hepatic hexokinase and glucose-6-phosphate dehydrogenase in the liver and increasing glucose uptake by peripheral and muscle tissues. Secondly guava leaf extract reduces serum glucose level by inhibiting glucose absorption from the small intestine. The latter effect is mediated by the inhibition of the intestinal enzymes responsible for the digestion of carbohydrates. This strategy represents one of the promising candidates used to control serum glucose level.

Glibenclamide works by binding to and inhibiting the ATP-sensitive potassium channels (KATP) inhibitory regulatory subunit sulfonylurea receptor 1 in pancreatic beta cells. This inhibition causes cell membrane depolarization, opening voltage-dependent calcium channels. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release [15]**.**

The present data indicate that the hypoglycemic efficacy of guava leaf extract alone was more potent than glibenclamide alone. This could be due to the two previously mentioned mechanisms of hypoglycemic effect of guava extract. However, the control-like level of serum glucose obtained when the rat model of diabetes was treated with guava leaf extract and glibenclamide could be attributed to the synergistic effect of the two agents which act by two different mechanisms.

It has been reported that oxidative stress may play an important role in the etiology of diabetes and its complications, such as vascular complications, diabetic cataract and diabetic nephropathy [16]**. Chen and Yen** [16] demonstrated that guava leaf extracts displayed a significant scavenging ability on the peroxyl radicals. The antioxidant capacity of this plant is attributed to its content of various phytochemical components, such as flavonoids, phenolic acids and carotenoids**.** The antioxidant efficiency of guava leaf extract could have a potential role in preserving the pancreatic β-cells by preventing their damaging by free radicals evolved by SZT. This in turn may represent one of the mechanisms by which guava leaf extract induces its antihyperglycemic effect [17].

The present results showed that the SZT-induced rat model of diabetes was associated with a significant decrease in weight gain. **Frier and Fisher** [18] stated that insulin deficiency causes lipolysis and proteolysis that result in weight loss. This is because insulin is not only a glucose lowering hormone but it is also a fat sparing hormone. Insulin is responsible for storage of excess calories in the form of body fat**.** Without insulin, the cells are not able to use glucose which the body prefers as a fuel. There is an increase in lipolysis from fat cells as well as protein breakdown, an exaggeration of the normal fasting state designed to provide alternative sources of fuel. Insufficient insulin prevents the body from getting glucose from the blood into the body's cells to use as energy. When this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight. These mechanisms, along with the caloric loss from glucosuria could underlie the weight loss reported in the present study.

When the present rat model of diabetes was treated with guava leaf extract, glibenclamide or their combination, the decrease in weight gain was restored to control-like value. This effect was associated with the improvement in the level of insulin. The increase in insulin level will save the required glucose, as a source of energy, for cells to get their caloric needs. Consequently, the processes of proteolysis and

lipolysis will be reduced. This could explain the returning of weight gain to its normal level.

In diabetic patients an impaired liver functions and kidney functions have been reported. Because STZ enters the cell via GLUT2, the toxic action is not specific to β cells and can cause damage to other tissues including the liver and kidney**.** STZ has well-known adverse side effects, which include hepatotoxicity and nephrotoxicity**.** There is evidence that STZ causes both direct organ toxicity and diabetes, and both actions can affect many organs including the liver [(19)]**.**

In the present study rat model of diabetes induced by streptozotocin was associated with increased activities in AST, ALT and alkaline phosphatase (ALP) and increased level in serum bilirubin. All these changes are indicators of impaired liver functions**.**

The liver is among the primary organs susceptible to the effects of hyperglycaemia-induced oxidative stress, which may lead to liver tissue injury**.** This is followed by derangement of protein, carbohydrate and lipid metabolism, thereby leading to increased oxidative stress and further triggering the inflammatory cascade**.** There is accumulating evidence especially in diabetic animal models such as streptozotocin-induced diabetic rats and mice that hyperglycemia induces acceleration of hydroxyl radical generation and this has been correlated with the level of thiobarbituric acid – reactive compounds**.** On the other hand, it was established that hyperglycemia increases mitochondrial reactive oxygen species (ROS) production, which could represent a key event in the development of diabetes complications**.** The initial cellular response to high glucose challenge is the generation of ROS, which rapidly induces apoptotic cell death [(20)]**.** Thus, the increase in aminotransferases activities may be due to the cellular damage in the liver caused by STZ induction.

The daily treatment of diabetic rats with guava leaf extract restored the activity of AST and ALT to nearly control-like values. Glibenclamide increased significantly AST activity more than the control and diabetic values and improved ALT activity from 253.1% to 27.8%. The combined treatment of diabetic rats with guava leaf extract and glibenclamide returned AST activity to control-like value and improved ALT to 23.2%.

The present data indicate that guava leaf extract has a potent hepatoprotective effect. This was evident from the ability of this extract to restore the increased AST and ALT induced by SZT to nearly control values. This effect could be attributed to the antioxidant effect induced by this plant [(16)]**.** Therefore, guava leaf extract may stabilize the cell membrane and prevent the leakage of AST and ALT to the blood stream. This effect could explain the restored AST and ALT activity. On the other hand, glibenclamide treatment showed a mild improvement in the liver function but failed to restore it to control values. This effect was concomitant with the improved glucose level. Thus, glibenclamide reduces the burden on the liver arising from its high activity to uptake glucose from blood stream. In addition, the antioxidant activity of glibenclamide was not proved. The persisted significant increase in AST activity that was observed after glibenclamide treatment may be due to its release from tissues other than the liver. The highest activities of AST are present in liver, cardiac tissue, and skeletal muscle, with smaller amounts present in the kidneys, pancreas, and erythrocytes. ALT, however, is predominantly present in the liver [(21)]**.** The improved activity of ALT that was observed after treatment with guava leaf extract, glibenclamide and their combination is an indicator of the improved liver functions. The altered ALP activity which is used for the assessment of liver function may reflect an increased hepatic insulin resistance or oxidative stress**. Levinthal and Tavili** [(22)] showed that STZ induced increased activity of ALP. The increased activity of ALP has been reported in pathological conditions involving the kidneys and liver**.**

Thus, the hepatoprotective effect of guava leaf extract mediated by its antioxidant effects may help in the improvement of ALP activity. The present data indicated that the reduction in ALP activity was more prominent with guava leaf extract than glibenclamide. Moreover, the present restoration of ALP activity to control-like value with the combined treatment may be due to the synergistic effect between guava leaf extract and glibenclamide.

The study of **Watkins *et al.*** [(23)] ascribed the increased level of bilirubin in SZT-induced rat model of diabetes to the enhanced hepatic hemoprotein catabolism. Data from long-term diabetic rats indicated that cytochrome P-450 is markedly reduced and it has been suggested that the decreased insulin levels could account for increased heme oxygenase activity in rat liver**.** The restoration

of bilirubin level to nearly control-like values by guava leaf extract, in the present study, could be mediated by improving the insulin level that prevents the catabolism of cytochrome P-450. In addition, the present data confirm the hepatoprotective effect of this plant through its antioxidant effect. Unfortunately, glibenclamide treatment failed to restore the increased bilirubin level to its normal value. However, the combined administration of guava leaf extract and glibenclamide reduced bilirubin level.

As clear from the present findings, the treatment of diabetic rats with guava leaf extract, glibenclamide or their combination improved renal functions. This was indicated from the improvement in creatinine level, normalized urea levels and reduced uric acid level induced by guava leaf extract

Al-Musa and AL-Hashem[24] observed morphological changes and elevated levels of urea and creatinine in diabetic kidneys. They suggested that these changes in kidney were due to extensive oxidative damage to renal tissue in the experimental diabetic animals. Also, observed that SZT could induce diabetes (hyperglycemia) and hypoinsulinemia and alters various metabolic and enzymatic functions of kidney resulting in various pathologic lesions. **Udemezue *et al.*** [25] observed that guava leaf extract induced a nephroprotective effect**.**

The improvement in renal function observed in the present study could be attributed to the ability of guava leaf extract, glibenclamide and their combination to improve serum glucose level. In addition the antioxidant activity of guava leaf extract may be additional factor role in improving renal functions.

CONCLUSION

It is clear from the present data that guava leaf extract possesses a more potent antihyperglycemic effect in comparison to glibenclamide, the well-established antidiabetic agent. This effect was indicated from the ability of the extract to restore the increase in serum glucose level to normal values. The potent antihyperglycemic effect of guava leaf extract could be due to its ability to promote glucose uptake by liver and other peripheral tissues together with the inhibition of intestinal glucose absorption induced by guava leaf extract. In addition, the antioxidant effect of guava leaf extract may have a role in preserving the β-cells and preventing further loses and inhibiting the pathogenesis of diabetic complications arising from free radical production. The present data showed also that guava leaf extract could minimize the impairment in hepatic and renal functions associated with diabetes.

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