Evaluation of Antioxidant Activity of *Psidium guajava* Linn. in streptozotocin–Induced Diabetic Rats

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ABSTRACT

Background: The incidence of diabetes is increasing rapidly in world wide. It produces disturbances in the metabolism of carbohydrate, protein, and lipid. Evidences recommended that the natural medicines originating from plant source may represent a culturally relevant complementary treatment for diabetes. **Objective:** The aim of the present study is to investigate the antioxidant activities of the ethanolic leaf extract of *Psidium guajava* Linn. in streptozotocin (STZ) induced oxidative stress in rats. **Methods:** Oxidative stress is induced with a single dose of STZ 60 mg/kg b.w. and then the animals are treated with a dose of various concentrations of ethanolic leaf extract of *P. guajava* (100 mg/kg b.w, 200 mg/kg b.w, and 300 mg/ kg b.w) for 45 days. After the treatment, the glucose, lipid peroxides (LPO), reduce glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), urea and creatinine levels are determined. Glibenclamide is used as a standard drug (3 mg/kg b.w.). **Results:** The present study exposed that the administration of ethanolic leaf extract of *P. guajava* showed a significant decrease in glucose and LPO levels. The treatment also finds that the significant increase in GSH, GPx, SOD and CAT levels in the liver, when compared with diabetic control rats. **Conclusion:** The results proved that the ethanolic leaf extract of *P. guajava* treated group may effectively regulate the antioxidant status in STZ induced diabetic treated groups.

Key words: Catalase, Glutathione peroxidase, Lipid peroxides, *Psidium guajava*, Reduce glutathione, Superoxide dismutase.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by persistent hyperglycemia with the disturbances of metabolism of carbohydrate, fat and protein. The metabolic changes related with diabetes cause secondary pathophysiologic changes in various organ systems that inflict a tremendous trouble on the individual with diabetes and on the health care system.[1] According to the statistics, in every 5 seconds someone is detected with diabetes and in every 10 seconds someone dies with diabetes in worldwide.[2]

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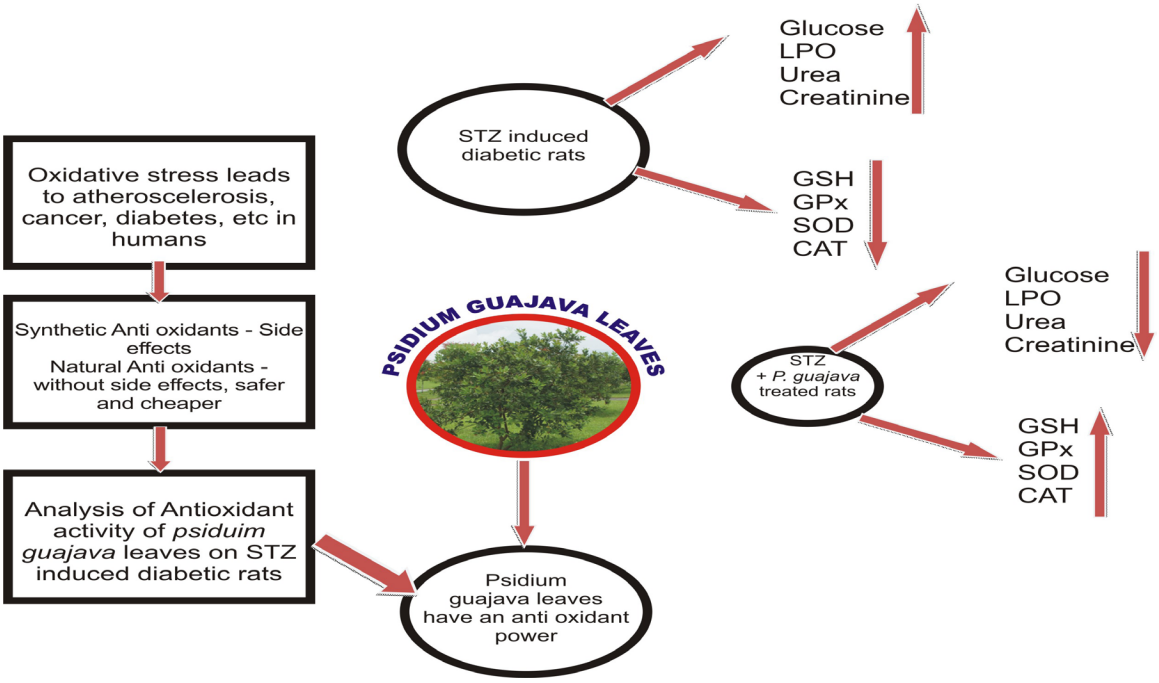
DOI: 10.5530/fra.2016.1.9

The oxidative stress, which plays an important role in chronic complications of diabetes mellitus. The free radicals such as superoxide anions, hydrogen peroxide, peroxynitrite and nitric oxide, which initiated the oxidative stress and these involved in the cellular damages. Our body cells have a protective mechanism against the free radicals and oxidative damages.[3] These mechanisms are disrupted in various pathological conditions and thereby the cellular damages are formed and it causes the various diseases like atherosclerosis, myocardial infarction and carcinogenesis.[4] The synthetic antioxidant agents are commercially available but these causes the toxic effect to human beings and animals.[5]

There is a growing interest in herbal medicines because of their efficiency and negligible side effects. The plant and plant extracts have good antioxidant activities due to the presence of various chemicals such as phenols, flavonoids,



Graphical Abstract

proanthrocyanidins and flavonols.[6] *Psidium guajava* Linn. is an important plant in the Myrtaeceae family. It has grown in all subtropical areas of the world, but the native of *P. guajava* is Mexico.[7] *P. guajva* have been reported to various activities like antimalarial,[8] antidiarrhoeal,[9] antimycobacterium[10] and antibacterial.[11] Leaves of this plant contains several compounds like various terpenoids,[12] phenols,[13] flavones,[13] flavonoids[14] and tannins.[15] Alloxan and streptozotocin (STZ) are toxic glucose analogues. The present study is aimed to evaluate the antioxidant activity of ethanolic extract of *P. guajava* in STZ induced diabetic rats and to establish its therapeutic potential in the treatment of diabetes and its complications.

MATERIALS AND METHODS

Plant material and extraction

The fresh *P. guajava* leaves are collected locally and authenticated by Botanist at Rapinat Herbarium, St. Joseph College, Trichy, Tamil Nadu, India. The extraction of *P. guajava* leaves are done by a hot percolation method with Soxhlet apparatus. Ethanol is used as a solvent. About 100 gm of the powder of the plant materials is extracted with 600 ml of ethanol. The extract is concentrated to dryness under controlled temperature of 40–50°C. The percentage of yield is found to be 10.15%.

Animals

Male albino rats of 6–8 weeks age, weighing 150-180 g is used. The animals are kept in clean, dry plastic cages and fed with standard pellet diet and water. This study is carried out in the animal house of Srimad Andavan Arts and Science College, Trichy (CPCSEA approval No–790/03/ac/ CPCSEA) and this study is approved by the Institutional Ethical Committee. The animals are divided into six groups with six rats each.

Group I: Normal rats (saline 2 ml/kg body weight).

Group II: Diabetic control rats (STZ induced as 60 mg/kg b.w).

Table 1: Levels of LPO, GSH, GPx, SOD and CAT in liver tissues of control and experimental group of rats

Treatment Groups	LPO (n mol/ g)	GSH (mg/g)	GPx (µ mol/min/mg)	SOD (µmol/min/mg)	CAT (µmol/min/mg)
Group I	2.15 ± 0.31	18.23 ± 0.15	26.15 ± 0.35	11.15 ± 0.24	26.42 ± 1.52
Group II	4.92 ± 0.64	7.15 ± 0.24	11.35 ± 0.41	4.56 ± 0.48	12.10 ± 0.52
Group III	4.24 ± 0.45	11.15 ± 1.09	13.52 ± 0.74	6.74 ± 0.11	14.15 ± 1.20
Group IV	3.85 ± 0.13	13.58 ± 1.89	18.15 ± 0.84	8.13 ± 0.52	16.38 ± 0.48
Group V	2.97 ± 0.22	15.26 ± 0.74	22.14 ± 1.23	9.72 ± 0.45	21.85 ± 0.42
Group VI	2.43 ± 0.45	16.85 ± 0.91	23.10 ± 0.87	10.15 ± 0.36	22.15 ± 0.94

Table 2: Levels of blood glucose, urea and creatinine level in control and experimental group of rats

Treatment groups	Blood glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Group I	86.9 ± 1.01	18.73 ± 0.30	0.85 ± 0.02
Group II	250.13 ± 3.21	70 ± 0.58	1.93 ± 0.015
Group III	179.42 ± 2.65	59.83 ± 0.60	1.62 ± 0.012
Group IV	143.62 ± 2.13	49 ± 0.58	1.44 ± 0.009
Group V	94.42 ± 3.16	34.33 ± 0.33	1.01 ± 0.018
Group VI	87.68 ± 2.14	30.33 ± 0.88	0.92 ± 0.012

Group III: Diabetic induced animals are fed with plant extract for 45days (100 mg/kg b.w).

Group IV: Diabetic induced animals are fed with plant extract for 45days (200 mg/kg b.w).

Group V: Diabetic induced animals are fed with plant extract for 45days (300 mg/kg b.w).

Group VI: Diabetic induced animals are fed with standard drug glibenclamide (3 mg/kg b.w).

Biochemical Analysis

The antioxidant status is assessed in the liver of the experimental rats. A known weight of the tissue was homogenized in 0.1 M ice cold tris–HCl buffer (pH 7.5) to give a 10% homogenate and used as the source of antioxidants. The levels of lipid peroxides (LPO),[16] reduce glutathione (GSH),[17] glutathione peroxidase (GPx),[18] superoxide dismutase (SOD),[19] and catalase (CAT),[20] blood glucose,[21] urea[22] and creatinine[23] are estimated at the end of the study (45th day).

Statistical analysis

Statistical analysis is carried out by using one way ANOVA as in Standard Statistical Software Package of social science (SPSS) version 12.0. P values <0.05 are considered as level of significance.

RESULTS

Table 1 shows the concentration of LPO, GSH, GPx, SOD and CAT in the liver of normal control and experimental groups of rats.

The antioxidant activity of *P. guajava* leaf extract is shown in Table 1. The concentration of LPO is significantly higher in STZ treated rats (group II), as compared to normal control animals (group I). These constituents are found to attain a near normal level in STZ plus *P. guajava* (group V), treated rats. The values are similar to the standard drug glibenclamide treated animal (group VI).

The concentration of GSH, GPx, SOD and CAT is significantly decreased in STZ treated rats, as compared to normal control animals. These constituents are found to attain a near normal level in liver of *P. guajava* treated rats in group IV and V. In group III there is slight but significant changes are noted and in group V, these values are nearer to the standard drug treated group.

The level of blood glucose, urea and creatinine levels are shown in Table 2. The level of blood glucose, urea and creatinine levels are increased in group II as compared to normal control animals. The concentration of blood glucose, urea and creatinine levels are found to attain a near normal level in group V than group III and IV when compared with the standard drug.

DISCUSSION

Hyperglycemia induces the formation of free radicals, and it affects the cellular functions. The membranes are damaged and it increases the level of lipid peroxidation.[24] The lipid peroxidations are highly reactive and it leads to damage the protein and DNA, finally causes the various diabetes mediated complications.[25] The degree of tissue damage influenced by free radicals, depends on the balance between the generation of free radicals and the endogenous antioxidant resistance mechanism.[26] In the present study, the LPO levels are increased in the STZ induced diabetic rats, and the levels are reduced after the treatment of ethanolic extract of *P. guajava* treated group. These values are similar to the standard drug treated group.

In the present study, the level of GSH is decreased in the diabetic treated group, and the levels are normal at the ethanolic extract of *P. guajava* treated groups, and this extract treated group values are similar to the standard drug treated group. GSH is the intracellular free radical scavenger. It plays a vital role in the maintenance of plasma antioxidant status and it is the cofactor for several enzymes. Reduced GSH level is decreased in many models of diabetes. Reduced GSH level is decreased in the mesangial cells[27] and muscle cells[28,29] when exposed to high concentration of glucose. A decreased GSH content may predispose the cells, to lower

the defense activity against the oxidative stress during the disease conditions including diabetes.[25]

In the present study, the level of GPx is lowered in the STZ treated group and the levels are increased in the *P. guajava* ethanolic extract treated groups. The activities of plant extract treated groups are similar to drug treated group. GPx is an enzyme that destroys the peroxides and it plays a vital role to provide an antioxidant defenses to an organism. GPx involved in the elimination of hydrogen peroxide.[30] GPx lowering the activity, the LPO are accumulated and increased the oxidative stress in diabetic rats.[31]

In diabetic rats, the levels are significantly reduced and the levels are regained after the treatment of *P. guajava* ethanolic leaf extract treated groups. The levels are similar to the standard drug treated group levels. SOD is one of the most important enzymes in the antioxidant defense mechanism.[32] It removes superoxide anion, it converts the superoxide anion into hydrogen peroxide and it retards the toxic effects caused by this radical. The lowering activity of this enzyme induces the oxidative stress in diabetic rats.[31]

Several studies have exposed that the lower antioxidant had improved peroxidative status in diabetes mellitus.[33,34] CAT is an antioxidant enzyme which is present in the tissues. It plays a vital role in the decomposition of the hydrogen peroxide molecule, and it prevents the tissues from the reactive hydroxyl radicals.[35] STZ inhibits the activity of CAT. Inhibition of this enzyme may induce the formation of hydroxyl radical and it damages the cell. In the present study, in the STZ treated rats the level of the CAT enzyme is reduced and the levels of this enzyme are increased in the ethanolic leaf extract of *P. guajava* treated groups and this level is similar in the standard drug treated group.

The STZ which destruct the beta cells of the islets of Langerhans, it reduces the insulin secretion, and it induces the hyperglycemia by the increasing the blood glucose level.[36] In the current study the oral administration of ethanolic extract of *P. guajava* leaves (100, 200, 300 mg/kg body weight) caused a significant reduction in the blood glucose level when compared to untreated diabetic rats.

Out of the three forms (100, 200, 300 mg/kg body weight), *P. guajava* 300 mg/kg seems to be the best antioxidant activity against STZ induced hyperglycemia, and it has been effective as the standard antidiabetic agent, this may be due to the synergistic effect of the different types of secondary metabolites present in it.

Administration of the *P. guajava* extract showed a significant decrease in blood glucose level. It also showed an improved antioxidant potential as evidenced by decreased LPO and a significant increase in the activity of CAT, SOD, GPx and GSH.[37] The present study is also accordance with the previous study.

The urea and creatinine are significant markers of renal function, this level is elevated in the diabetic hyperglycemias conditions.[38] In the present study the levels of urea and creatinine are increased in the STZ treated groups, the represents the imparted renal function. The levels of urea and creatinine are reduced in the plant treated groups. This may support the *P. guajava* leaves prevent STZ induced biochemical alterations and does not cause renal damage.

CONCLUSION

The results proved that the ethanolic extract of *P. guajava* may effectively regulate the antioxidant status in STZ induced diabetic rats. It may be the reason for its hypoglycemic property. On the basis of these findings and other scientific evidences supports *P. guajava* may be used to treat various ailments due to antioxidant imbalance.

ACKNOWLEDGMENT

The authors are grateful to The Chairman, M.I.E.T. Institutions, for his guidance and encouragement.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ABBREVIATION

CAT: Catalase
GPx: Glutathione peroxidase
GSH: Reduce glutathione
LPO: Lipid peroxides
SOD: Superoxide dismutase
SPSS: Standard Statistical Software Package of Social Science
STZ: Streptozotocin

Highlights of the paper

- This article highlights some of the recent scientific support for the antioxidant potential of *Psidium guajava* in diabetic induced animal models.

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