Research Article

Antidiabetic and ameliorative efficacy of two varieties of *Psidium guajava* L. fruit extracts in type 2 diabetes and indices of complications in diabetic albino rats

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ABSTRACT

Background: Diabetes mellitus (DM) is a chronic metabolic disease which leads to dysfunction in the insulin production/insulin action or both. Hyperglycemia condition in DM results in oxidative stress with decreased antioxidants which leads to various diabetes-related complications. Natural antioxidants are considered to be very effective in preventing hyperglycemia due to the presence of chemical constituents in it. **Aims and Objectives:** This study aims to investigate the antidiabetic and ameliorative efficacy of two varieties of *Psidium guajava* L. (PG), namely, Lalit (LA) and *Allahabad safeda* (AS) in preventing complications in type 2 diabetes in albino rats. **Materials and Methods:** Matured male albino rats were randomly divided into four groups of six animals ($n = 6$) each. The first group of rats was served as control. The second group of rats was injected with 45 mg/kg/bw of streptozotocin (STZ) intraperitoneally for the induction of diabetes. The third and fourth groups of STZ-induced rats were supplemented with 200 mg/kg/bw of fruit extract of LA and 400 mg/kg/bw fruit extract of AS through oral gavage for 8 weeks, respectively. **Results:** Significant elevation in lipid peroxidation (LPO) levels and reduced activities of superoxide dismutase, catalase, reduced glutathione (GSH), glutathione peroxide (GPx) were observed in pancreas tissue of diabetic group in contrast to control group. However, except the activity of GPx, treatment of 200 mg/kg/bw of LA significantly prevented the above changes. In comparison, the fruit extract of AS (400 mg/kg/bw) could not prevent diabetes-induced alterations in antioxidant enzymes and LPO levels. **Conclusion:** Our findings clearly indicate, fruit extract of LA of PG is potent enough to prevent oxidative stress in diabetic rats compared to AS. Thus, LA fruit extract could be used as natural antidiabetic in DM.

KEY WORDS: Antioxidant; Pancreas, *Psidium guajava* L., Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease, which have attained epidemic distributions globally, among human population. According to 2017 survey, DM affected almost 450 million people of age group of 19–99 years which is almost 8.40% of world's total population. The pervasiveness might emerge up to 9.90% approximately 693 million of world's population within the year 2045.[1] DM leads to persistent hyperglycemia, distinguished by abnormalities in carbohydrate and protein metabolism due to relative inadequacy of insulin production or insulin action.[2] Clinical studies demonstrated that early stage of hyperinsulinemia due to insulin resistant, later transferred gradually to late stage process called hypoinsulinemia which is a secondary cause to diminish the function of beta cells of pancreas.[3] Hyperglycemia is a source of high generation of reactive oxygen species (ROS) from glucose auto-oxidation,[4] protein glycosylation[5] which leads to oxidative stress. Oxidative

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stress is linked with elevation in ROS levels with reduced levels of antioxidant enzymes. The ROS-mediated induced cell injury and decreased insulin secretion lead to diabetes-related complications.[6]

Streptozotocin (STZ) has been extensively used in inducing diabetes in experimental animals, as it leads to deterioration of beta cells of pancreas analogous to those found in diabetic human.[7] STZ is a well-known diabetogenic agent which exerts its detrimental effect, leading to destruction or dysfunction of beta cells of pancreas by enhancing oxidative stress and apoptosis with reduced antioxidant defense mechanism.[8] Since DM and its complications are a major health issue worldwide, there is a greater interest in the field of medicine for its prevention. Till date, numerous researches have been done to cure diabetes, yet outcome is not adequate. Hence, it is a right moment to move on to a new alternative approaches. In this line, the herbal products are considered as better alternative medicine because of their negligible inimical effects with less price in contrast with synthetic drugs.[9]

Fruits have long been contemplated as a rich source of antioxidants. In recent days, antioxidant potential of fruits has received a greater emphasis in maintaining the levels of oxidative stress. The current study is based on important Indian herb, *Psidium guajava* L. (PG) belongs to the family Myrtaceae, fruit extract of PG is abundant in Vitamin C and it contains copious amount of antioxidants and polyphenols.[10] Cheng *et al*. and Gutiérrez *et al*. reported that the presence of flavonoids and polyphenolic compounds in PG plays a key role in antidiabetic properties through its free radical scavenging capacity.[11,12]

Although there are ample of studies, there is no such comparative studies found, to assess the potentiality of different varieties of fruits of PG in prevention of oxidative stress in DM. With this background, the current study was undertaken to analyze the ameliorative efficacy of two varieties of PG that is Lalit (LA) and *Allahabad safeda* (AS) in preventing complications in type 2 diabetes in STZ-induced albino rats.

MATERIALS AND METHODS

Animals

Matured male albino rats of Wistar strain (180–200 g) were procured from Sri Raghavendra Enterprises, Bengaluru. Animals were maintained under constant normal laboratory conditions (12 h light:12 h dark) without any disturbances. During the experimental period, rats were fed with standard rodent pellet food and provided water *ad libitum*. All the experimental protocols were complied with National Institution of Nutrition, Hyderabad (Guidelines for the Care and Use of Laboratory Animals), and were approved by (IAEC No.402/01/a/CPCSEA) Bioethics Committee of Faculty of Zoology at Bangalore University, Bengaluru.

Chemicals

Streptozotocin and epinephrine were obtained from Sigma-Aldrich Ltd., Bengaluru, India. Glutathione (GSH), 5,5-Dithio-bis-2-nitrobenzoic acid, and 1-chloro-2,4-dinitrobenzene were obtained from Merck India Ltd., Mumbai. The analytical grade of bovine serum albumin and other chemicals was obtained from SD Fine Chemicals Ltd., India, and SISCO Research Laboratories, India.

Collection and Preparation of the Extract

Two fruit varieties of *Psidium guajava* L., namely, Lalit (LA) and Allahabad safeda (AS) were collected from Regional Horticultural Research and Extension Centre, University of Horticulture Science, Bengaluru. Fresh fruits of LA and AS were washed with tap water, rinsed with distilled water, and chopped. The chopped fruits were desiccated for about 45–50 days and pulverized using a mechanical blender.

Later, the powdered extracts were subjected to Soxhlet extraction separately using different solvents with increasing polarity. Ethanolic extract was selected for the assay and it was concentrated to dryness using a rotary evaporator.

Induction of Diabetes

STZ was injected intraperitoneally to male rats with a single dose of 45 mg/kg/b.w. After 72 h of STZ injection, fasting blood glucose levels were examined (Accu-Chek Active Glucometer) for the confirmation of induction of diabetes. STZ-induced rats showing fasting blood glucose levels above 220 mg/dl were regarded as diabetic and used for the experimental studies.

Experimental Design

Twenty-four rats were randomly divided into four groups of six animals each ($n = 6$). The first group of rats was served as control. The second group of rats was STZ induced (45 mg/kg/b.w), and the third and fourth group rats were STZ induced and supplemented with 200 mg/kg/bw of LA and 400 mg/kg/bw of AS fruit extracts, respectively, for 8 weeks. After the treatment period, all the rats were euthanized with 1% pentobarbital sodium and sacrificed through spinal dislocation and pancreas was removed and homogenized, centrifuged and supernatant was used for biochemical assays.

Estimation of Biochemical Markers

The concentration of the malondialdehyde was done according to the method.[13] Superoxide dismutase (SOD) activity was done by the method.[14] Protein estimation was done by following the protocol of Lowry *et al*.[15] The activity of catalase (CAT) was done by the procedure.[16] The levels of reduced GSH was followed by the method.[17] The levels of GSH peroxidase (GPx) were done by the method.[18]

Statistical Analysis

Results were expressed in Mean±SEM. Statistical analysis was performed using one-way analysis of variance followed by Duncan's multiple range test using SPSS software and judged significance if $P < 0.05$.

RESULTS

There was a significant increase in lipid peroxidation (LPO) of STZ group alone as well as fruit extract of AS supplemented group in contrast with control group. However, the LPO levels in LA extract supplemented group were alike in contrast with control group [Figure 1a].

The activity of SOD decreased in diabetic groups collated with controls. However, there was no dissimilarity in the levels of SOD between a control and LA supplemented groups. In contrast, the SOD activity of AS supplemented group showed significant similarity with STZ-induced groups [Figure 1b].

The activity of CAT was significantly reduced in STZ group contrast to control group. However, there was no difference in the levels of CAT between LA supplemented rats and control rats. In contrast, the CAT activity of AS-treated diabetic rats was significantly greater than STZ-treated rats and lesser than that of controls [Figure 1c].

GSH levels were significantly reduced in STZ group and AS-treated group in contrast to control group. Supplementation of LA extract showed greater levels of GSH and found to be similar in contrast to control group [Figure 1d].

The activity of GPx was significantly reduced in diabetic groups in contrast with control group. Nevertheless, the levels of GPx in LA and AS supplemented rats were significantly elevated than STZ group and lower in contrast with control group [Figure 1e].

DISCUSSION

The present investigation analyzed the ameliorative efficacy of two different varieties of fruit extracts of PG in type 2 diabetes and indices of complications in diabetic albino rats results revealed that LA was more potent as antidiabetic agent compared to AS.

Severely damaged β cells cause deterioration in the activity of insulin activity which leads to DM. Earlier studies suggest that impairment in insulin secretion and associated hyperglycemia is due to oxidative stress.[19] Sustained hyperglycemia elevates ROS levels in pancreas in turn causing oxidative stress.[20] LPO is a chief biological marker of oxidative stress.[21] The present investigation showed, increased LPO in pancreas with concomitant reduced levels of antioxidant activities in STZ rats due to elevated ROS levels. Destruction of beta cells plays a principal role in the production of free radicals in diabetes-induced rat pancreas and the alterations can be identified by determining LPO levels in pancreas. Upraised activity of oxidative stress in STZ-induced rats might due to glucose autoxidation, elevated lipid peroxidation, and reduced levels of antioxidant activities.[22,23] In the present study, significant elevation of LPO levels was noticed in diabetic group in contrast with control group. However, oral supplementation of LA extract significantly prevented the elevation of LPO in diabetic rats.

Further, antioxidant defense system showed impairment in the pancreas of STZ group. It was evident with reduced levels of enzymatic and non-enzymatic antioxidants, namely, SOD, CAT, GSH, and GPx in diabetic rats compared to controls. Pancreatic β cells are vulnerable to ROS caused injury, programmed cell death,[24,25] and diminished secretion of insulin. Immoderate levels of ROS can persuade β-cells injury through intervention of intracellular signal transduction apart from direct oxidative damage.[26,27] In the current investigation, supplementation with fruit extract of LA normalized the levels of SOD, CAT, and GSH and the values are in contrast with controls. Bagri *et al*. reported, levels of SOD, CAT were elevated in PG extract treated STZ rats and this might be due to enriched antioxidant source in PG.[28] Our study is in line with the above report. It is known that stimulation of SOD levels were advanced by fruit extract of LA and it might have quicken the dismutation of superoxide to H_2O_2, in turn rapidly eliminated by CAT. This causes protection to the tissues against toxicity and consequently prevents the elevated LPO levels. The outcome of our study is in line with the previous reports.[29,30]

Furthermore, in DM group, the reduced levels of GPx activity might be due to augmented H_2O_2 production induced by oxidative stress.[31] Significant elevation in the levels of GPx in pancreas of PG supplemented rats indicates, enriched antioxidant source present in PG plays a key role in protecting the tissues from diminishing effects caused due to oxidative stress markers.

The another important findings of the present investigation are the comparative effect of two varieties of fruit extracts of PG. Vitamin C has been identified in protecting STZ-induced DNA damage in mice which could support in the management of diabetes.[32] The active compounds present in the PG fruit helps in hypoglycemia and restoration of enzymatic alterations.[33,34] Our studies are in line with the above said reports. Plants such as *Allium sativum*, *Gymnema sylvestre*, *Lagerstroemia speciosa*, and *Ficus benghalensis* have also shown antihyperglycemic and insulin release stimulatory effect.[35]

The fruit extract of LA was potent in preventing oxidative stress in STZ-induced rats in contrast with AS extract. Supplementation with LA normalized the levels of antioxidant

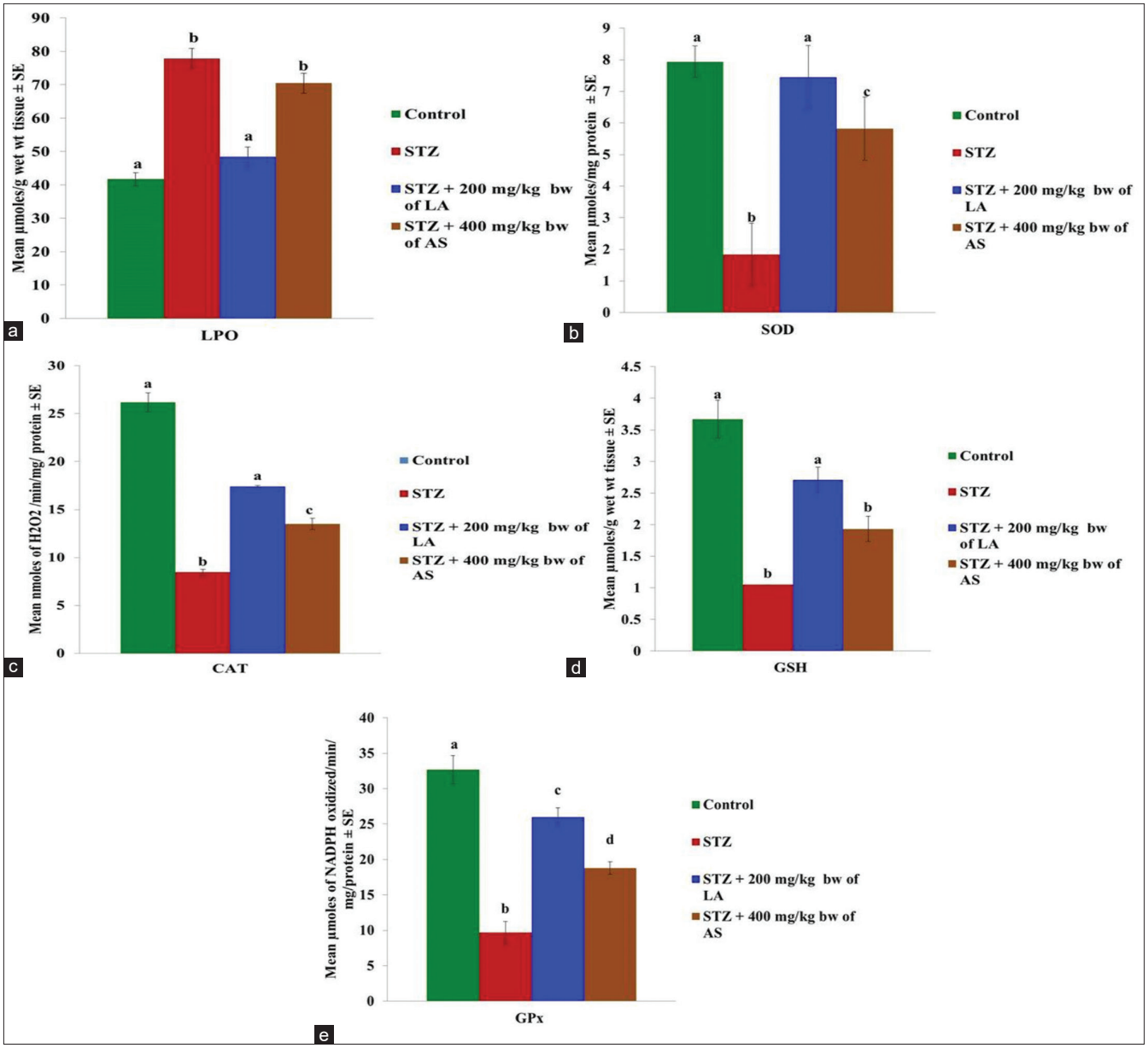

Figure 1: (a-e) Streptozotocin-induced oxidative stress and ameliorative efficacy of Lalit and *Allahabad safeda* extracts in pancreas tissue of rats. (a) Changes in lipid peroxidation content; (b) changes in SOD level; (c) changes in CAT activity; (d) changes in glutathione level; (e) changes in glutathione peroxides activity. Values are mean ± SE of six rats in each group; Alphabets "a, b, c, d" are significantly different among control and experimental groups ($P < 0.05$)

enzymes and LPO activity. However, treatment with AS extract could not normalize the variations in antioxidant enzymes and LPO levels in STZ rats.

Our results clearly indicate that fruit extract of LA possesses enriched antioxidants and antidiabetic properties. Thus, further studies in this line are required to isolate and study the properties of active compounds of LA.

CONCLUSION

It is inferred from the current investigation that between two varieties of PG, the fruit extract of LA was more potent in preventing complications in type 2 diabetes compared to AS. This is due to its antioxidant activity and hence the extract of LA could be used as natural antidiabetic component in DM.

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