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Protective Role of *Psidiumguajava* L. Leaf Extract in Preventing Diabetes Induced Oxidative Stress in Pancreas of Albino Rats

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Abstract:-The purpose of the current study is to evaluate the efficacy of *Psidiumguajava* L. (PG) leaf extract in preventing diabetes-induced oxidative stress in male albino rats. Induction of Streptozotocin (STZ) resulted in a significant elevation in lipid peroxidation levels in the pancreas of diabetic induced rats. In contrast, the activity of superoxide dismutase, catalase, and reduced glutathione activities were significantly decreased in STZ induced diabetic rats compared to controls. However, supplementation with 250mg/kg/b.w of PGleaf extract for eight weeks, significantly prevented the above alterations. In contrast, 500 mg/kg/b.w of leaf extract of PG could not prevent the STZ induced alterations in antioxidant enzyme activities in diabetic rats. These results clearly suggests the lower dose of PG leaf extract is potent enough to prevent oxidative stress in diabetic induced rats.

Keywords:-*PsidiumguajavaL.,Streptozotocin, Lipidperoxidation, Catalase.*

I. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabollic disorder affecting a considerable population worldwide. It is characterized by elevated glucose levels with a deficiency in insulin action or secretion or both. The diabetic complications *viz.*, micro-vascular and cardiovascular changes are due to oxidative stress. Oxidative stress in DM leads to βeta-cell dysfunction, diminished levels of glucose tolerance, and depletion in the levels of antioxidant enzymes, thereby causing detrimental effects on free radical scavenging activities [1].βeta-cells of pancreas are vulnerable to oxidative damage due to less antioxidant activity and surplus of reactive oxygen species (ROS) [2]. Antioxidants play a vital role in reducing the risk of diabetic complications by arresting the free radical destruction within the cell [3,4]. Streptozotocin (STZ) is used to induce diabetes in experimental studies, STZ causes toxicity mainly by elevating oxygen free radicals and thereby destroying the pancreatic βeta-cells and DNA damage in the cell [5]. Studies have been reported, STZ inhibits the activities of antioxidant enzymes *viz.* superoxide dismutase (SOD), catalase (CAT) and increases lipid peroxidation in diabetic rats [6,7].

Medicinal plants are the source of diverse nutrients and bioactive compounds that play a significant role in therapeutic applications. Scientific interest in medicinal plants has provided unlimited opportunities for new drug discoveries [8]. *Psidiumguajava* L. (PG) is an essential evergreen plant that belongs to the family Myrtaceae. The fruits and leaves of PG consist of enriched natural antioxidants *viz.*, vitamin C, and polyphenolic compounds [9, 10]. Due to its abundant source of antioxidant properties, the current experiment was designed to evaluate the potential role of PG leaf extracts against oxidative stress in STZ induced rats.

II. MATERIALS AND METHODS

A. Animals

Adult male albino rats (180–200g) were procured from Sri Raghavendra Enterprises, Bangalore. Rats were maintained under constant laboratory conditions(12hr light:12hr dark) without any disturbances. During the experimental period, rats were fed with standard rodent pellet food and provided with water *ad libitum.* All the experimental protocols complied with the National Institution of Nutrition, Hyderabad (Guidelines for the Care and Use of Laboratory Animals) and were approved by the Bioethics Committee of Faculty of Zoology at Bangalore University, Bengaluru.

B. Chemicals

Streptozotocin, Ellman's reagent, Epinephrine and Glutathione were purchased from Sigma Aldrich Ltd. Bangalore, India. The other chemicals of analytical grade were obtained from SD fine chemicals Ltd, India and SISCO research laboratories (SRL), India.

C. Preparation of leaf extract

Fresh leaves of *Psidiumguajava* L.(*Allahabadsafeda* variety) were washed with tap water and rinsed with distilled water and shade dried at normalroom temperature (27±2ºC) for 30-40 days. Leaves were powdered using a mechanical blender and subjected to soxhlet extraction using different solvents with increasing polarity. Ethanolic extract was selected for the assay and it was concentrated to dryness using a rotary evaporator.

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D. Induction of diabetes

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

III. EXPERIMENTAL DESIGN

Animals were acclimatized to laboratory conditions for one week prior to the experiment period. Twenty-four rats were randomly divided into 4 groups (n=6). The first group of animals was considered as controls, the second group consists of STZ induced diabetic rats. The third and fourth groups of rats were induced with STZ and orally treated with a high and low dose (250 and 500 mg/kg/bw) of PG leaf extracts for eight weeks respectively. After the treatment period, all the rats were euthanized with 1% pentobarbital sodium anesthesia and sacrificed through cervical dislocation and pancreas tissue was used for the biochemical assays.

A. Biochemical estimations

The lipid peroxidation(LPO) levels were done by determining thiobarbituric acid reactive substances by following the method of Niehaus and Samuelsson [11]. The amount of formation of MDA content was expressed as 'µmoles/g' and measured at 535 nm. The activity of CAT was done by the method of Aebi [12]. The decomposition of hydrogen peroxide (H_2O_2) was determined by absorbance at 240 nm and CAT activity was expressed as 'nmoles H_2O_2 consumed/min/mg protein'. The activity of SOD was assayed by determining the inhibition of epinephrine auto-oxidation by following the method of Misra and Fridovich[13]. The absorbance was recorded at the 30sec intervals of time for 4min at 480nm and results expressed in "units/mg protein." Reduced glutathione (GSH) assay was determined by the method of Ellman [14]. The amount of GSH was expressed as 'µ moles/ming/g'. Protein content was measured by following the method Lowry et al.,[15].

B. Statistical analysis

Values were expressed as Mean±SE. Statistical analysis were done by one-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT) and judged by significance if $P < 0.05$ (SPSS software).

IV. RESULTS

Significant elevation was observed in the levels of LPO in STZ induced diabetic rats compared to the control group. However, the activity of LPO in a lower dosage(250mg/kg/b.w) treated rats was similar to the control group. The activity of LPO of higher dosage (500mg/kg/b.w) treated rats did not show any significant variation when compared to STZ induced diabetic groups alone (Figure 1).

In contrast, a significant reduction was observed in the activities of SOD, CAT and GSH(Figure 2,3 and 4) in STZ induced rats compared to control rats. However, supplementation with a lower dosage of PG extract showed a significant increase in the activity of SOD, CAT, and GSH. Whereas activities of SOD, CAT, and GSH of high dosage of PG extract supplemented diabetic rats were similar to the results of STZ induced diabetic rats group.

Fig (1)

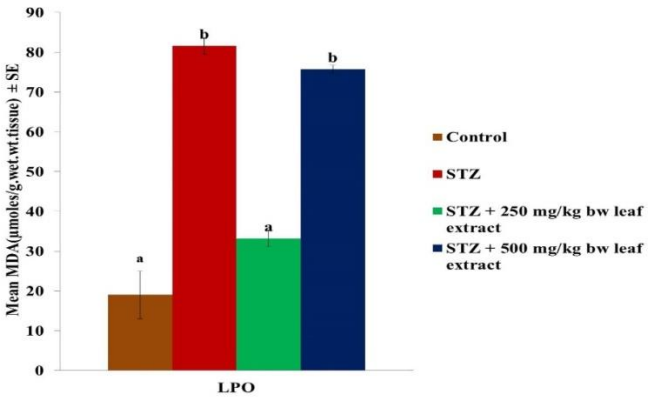

Fig (2)

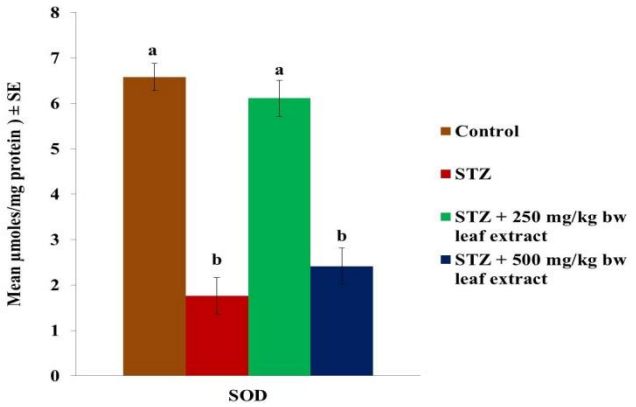

Fig (3)

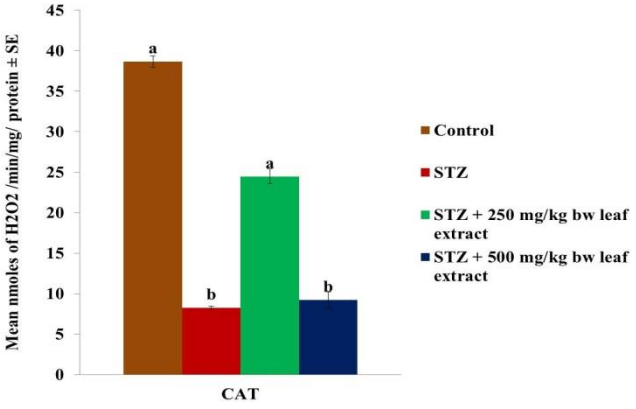

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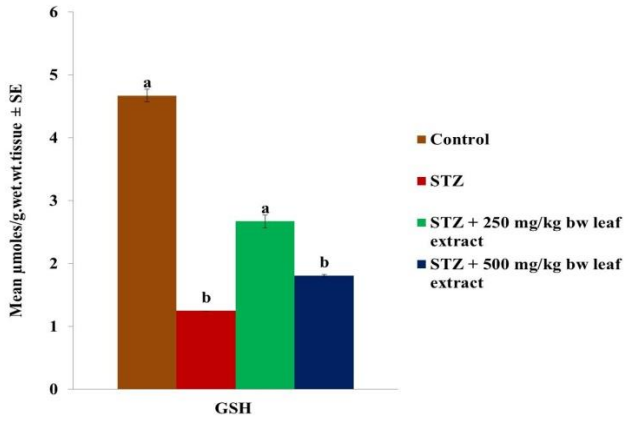
Fig (4)



Figures 1,2,3 & 4 represents the level of LPO, SOD, CAT and GSH contents in pancreas tissue of rats respectively. Values were represented in Mean±SE (n=6). Alphabets 'a' and 'b' represents significantly different among experimental groups as determined by DMRT at significance $P < 0.05$.

LPO-Lipid peroxidation, SOD- Superoxide Dismutase, CAT- Catalase, GSH – reduced glutathione, STZ-streptozotocin.

V. DISCUSSION

The present study revealed the preventive role of PG against oxidative stress in STZ induced diabetic rats. The cytotoxic effects of STZ is dependent on DNA alkylation by site-specific action with DNA bases and by free-radical generation [16,17]. The antioxidant enzymes *viz.*, SOD, CAT are the two major free radical scavenging enzymes. Superoxide dismutase detoxifies the superoxide anion by converting it into H_2O_2 and water. The CAT is a hemeprotein that catalyzes the reduction of H_2O_2 and protects tissues from hydroxyl radicals [18]. In diabetic condition, the activity of SOD declined due to its glycation under hyperglycemic conditions. The reduced activities of SOD and CAT in diabetic animals are due to the overproduction of ROS[19]. Similarly, reduced cellular GSH concentration and elevated lipid peroxidation are associated with diabetes.

Hyperglycaemia in DM induces oxidative stress which involved increased production of ROS. The enhanced free radical generation is due to a reduction in the antioxidant defense system which leads to cellular damage [20].In our current study, decreased activities of antioxidant enzymes and concomitant increase in LPO of diabetic rats compared to controls clearly indicates, enhanced production of ROS. Further, supplementation with leaf extract of PG normalized the levels of antioxidants and LPO levels, which clearly revealed the protective role of PG against oxidative stress in diabetes.

Further, between two different doses of PG extract, the lower dose i.e. 250 mg/kg/b.w was potent compared to that of higher dose i.e. 500 mg/kg/b.w. The activities of antioxidant enzymes and LPO of 250 mg/kg/b.w of leaf extract-treated groups did not significantly differ from controls. Similar activities were observed in antioxidant enzymes of control and 250 mg/kg/b.w of leaf extract treated groups. This could, clearly indicate that supplementation with a lower dose of leaf extract of PG restored the STZ induced alterations in the antioxidant system and LPO. These alterations might be due to free radical scavenging activities and anti-diabetic action, which is present in PG (*Allahabad safeda* variety) extract. The protective action of leaf extract of PG is due to its anti-hyperglycemic and antioxidant nature. Studies have shown that treatment with leaf extract of PG has significantly improved the insulin levels and hemoglobin in diabetic rats[21]. Leaves of PG consist of phenolic compounds, tannins, triterpenes, and flavonoids [22]. The presence of these compounds is partially responsible for its anti-diabetic activity. However, further studies are needed in this line to identify and isolate a potent anti-diabetic compound from the leaves of PG.

VI. CONCLUSION

The conclusion of the current study clearly indicates,*Psidiumguajava* L.leaf extract significantly prevented the oxidative stress in diabetic induced rats. Lower dosage of PG leaf extract 250mg/kg/b.w was potent enough in protecting pancreas against oxidative damage under diabetic condition.

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AUTHORSHIP STATEMENT

The experimental design of this study and guidance was given by Dr.UshaAnandhi. D and data analysis, writing, practical aspects of the study was carried out by Sowmya BH.

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