THERAPEUTIC ROLE OF ANTI-OXIDANT PROPERTIES OF *EMBLICA OFFICINALIS* (AMLA) IN STREPTOZOTOCIN INDUCED TYPE I DIABETIC RATS

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Summary

Objective: To find out anti-oxidant properties having therapeutic effectiveness in streptozotocin induced type 1 diabetic rats of fresh juice and various extracts of fruit of *Emblica officinalis* (Amla) gaertn, family – Euphorbiaceae.

Material and methods: Wistar rats of either sex were divided into four groups, six animals in each group. Group 1: normal control, Group 2: diabetic control, Group 3: diabetic rat treated with fresh juice, Group 4: diabetic rat treated with hydro alcoholic extracts of *Emblica officinalis* fruit. Diabetes was induced in rats by single i.v. tail vein injection of streptozotocin at dose of 45 mg/kg under light ether anesthesia. Control animals were injected with an equivalent volume of 0.9 % NaCl. Animal showing glucosuria (>2%) were considered as diabetic. At end of 30 days treatment animal were sacrificed by suitable method and liver were dissected out and subjected for estimation of tissue protein, super oxide dismutase, catalase, reduce glutathione and tissue malonadialdehyde levels.

Results: Diabetic animals showed significant increase in BSL, malonadialdehyde and decrease in superoxide dismutase, catalase and reduced glutathione levels in liver tissue compared to control groups. The results indicate potent antioxidant and lipid peroxidation inhibiting activities of fresh juice and hydro alcoholic extract of fruits of *E. officinalis* in diabetic rats.

Conclusions: Our data suggests, fresh juice and hydro alcoholic extract of fruits of *E. officinalis* possesses potential anti-diabetic and anti-oxidant activities in STZ induce type 1 diabetic rat. Our study also indicates link between oxidative stress and diabetes.

Key Words: Antioxidant, diabetes, *Emblica officinalis,* oxidative stress.

Introduction

Diabetes mellitus has been defined by American Diabetes Association Expert Committee in their 1997 recommendations as a group of metabolic diseases characterized by hyperglycemia, altered metabolism of lipids, carbohydrate and proteins resulting from defects in insulin secretion, insulin action of both. The chronic hyperglycemia is associated with long damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels thus covering a wide range of heterogeneous diseases. [1]

Diabetes is now recognizing as serious global health problem.

Oxidative stress is believed to initiate and aggravates many diseases including diabetes mellitus. In 1991, Baynes underlined role of oxidative stress in the evolution and progression of diabetes. [2] Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. [3] Therefore oxidative stress, antioxidant defense, cellular redox status should be regarded as the central players in diabetes and its complication.

The fruits of *Embelica officinalis* gaertn. commonly known as amla is known for its medicinal and therapeutic properties from ancient time in India and considered as a wonder fruit for health conscious population. Amla are widely used for their preventive, curative and health restorative properties.

Amla contains highest amount of vitamin c, low and high molecular weight tannins (30%), phyllembic acid (6.3%), phyllebin (2.4%), gallic acid and ellagic acid and cytokine like substance identified as zeatin, z-riboside and z nucleotide. The fruit contains 482.14 units of superoxide dismutase / g fresh weight, and exhibited antisenscent activity. [4] Vitamin c is a powerful water soluble antioxidant, neutralizing harmful reactions in blood and fluid inside surrounding cells. For people with diabetes vitamin C appears to protect against impaired glucose tolerance and other complications of diabetes. [5]

In light of above fact the present study was designed to investigate protective effect fresh juice and hydroalcoholic extracts of fruits of *E. officinais* on lipid peroxidation level and on antioxidant enzymes superoxide dismutase (SOD), reduced glutathione (GSH) and catalase in STZ induced type 1 diabetic rat and a preclinical study to define the correlation between diabetes and oxidative stress.

Materials and Methods

The fresh fruits of *Emblica officinalis Gaertn*., family Euphorbiaceae, were identified and authenticated by Prof. O. P. Saxena, Head, Botany department, Gujarat University, Ahmedabad, India.

Preparation of plant extracts:

Fresh juice preparation*:* Fresh fruits were cuts into pieces and seeds were removed. Fruits pieces were weighed and equal volume of water added and grind in mixer grinder. Filter the fresh juice with cotton clothes, thus juice obtained were used for treatment.

Hydro alcoholic extract preparation: Powder of shade dried fruits of *Emblica officinalis* was extracted exhaustively in round bottom flask with water and methanol mixture at 40-60^{o} C in proportion of 1:1 for 48 hours. The hydro alcoholic extract thus obtained was filtered and solvent removed under vacuum.

Animal*:* Health wistar rats of either sex weighing 150-200 gms were used for study. The animals were housed under well-controlled conditions of temperature (22$\pm$2^{o}C), humidity (55$\pm$ 5%) and 12h/12h light dark cycle. Animals were free access to conventional laboratory diet and tap water ad libitum. The protocol of experiment was approved by institutional animal ethical committee as per guidance of committee for purpose of control and supervision of experiments on Animals (CPCSEA), Government of India. Diabetes was induced with streptozotocin (STZ) (Sigma, USA) 45 mg/kg dissolved in 0.9 % NaCl, administered as a single i.v. tail vein injection under light ether anesthesia. Control animals were injected with an equivalent volume of 0.9 % NaCl. The experimental animals were divided into four groups, six animals in each group.

Group I: normal control rats

Group II: Diabetic control rats

Group III: Diabetic rats treated with fresh juice (5 ml/kg, p.o., per day)

Group IV: Diabetic rats treated with hydro alcoholic extracts (100 mg/kg, p.o., per day)

Animals were checked for the extent of glucosuria 48 hours after injection of STZ using diastix (Bayer Diagnostics, India). Animal showing glucosuria (>2%) were considered as diabetic. 5% glucose solution was given 2 days before and 3 days after STZ injection to prevent initial hypoglycemic effect of STZ.

At the end of 30 days treatment, blood sample were collected and subjected to estimation of BSL by Serum glucose estimation kit (Span Diagnostics Ltd., India) and after animal were sacrificed by spinal dislocation technique and liver was dissected out, rinse with ice cold distilled water followed by sucrose solution (0.25 M). It was blotted free of blooded and tissue fluids then weighed on analytical balance. Then it was cross chopped with surgical scalpel into fine slices and was placed in chilled sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in ice-cold 10 mm tris HCl buffer at a concentration of 10 % (w/v) with 25 strokes of tight Teflon pastel of glass homogenizer at a speed of 2500 RPM.

The prepared homogenates were centrifuged in cooling centrifuge (REMI cooling centrifuge) at 6000 RPM for 20 minutes; temperature was maintained at -5 to 1^{o} C during centrifugation. Clear supernatant was separated and used to estimate tissue protein levels, SOD, catalase, reduce glutathione (GSH) and tissue lipid peroxidation (malonadialdehyde- MDA) levels.

The liver tissue protein was estimated by the method of Lowry et al. [6] and takes standard as solution of crystalline bovine serum albumin. Lipid peroxidation product MDA was estimated as the method described by Ohkawa et al,. [7] Reduced GSH and SOD levels in tissue homogenates were estimated as per method described by Beutler et al. [8] and Mishra et al. [9] respectively. Decomposition of H_2O_2 in presence of catalase was estimated by Aeibi et al., 1976. [10]

Statistical analysis*:* statistical difference between means of various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at P value $\leq$ 0.05 and highly significant at P $\leq$ 0.001. Statistical analysis was performed using Sigma stat statistical software.

Results

As shown in table 1, STZ diabetic rats were found to exhibit significant increase BSL in blood and MDA levels in liver as normal control rats. Treatment with fresh juice and hydro alcoholic extract of fruits of *Embelica officinalis* produced significant decrease BSL in MDA level. The degree of reduction in BSL and MDA was greater with fresh juice as compared to hydro alcoholic extracts. STZ – diabetic rats were found to exhibit significantly decreased SOD and catalase enzyme levels in liver as compared to normal control rats. Treatment with fresh juice and hydro alcoholic extracts of fruits of *Embelica officinalis* produced significant increase in SOD and catalase levels in diabetic rats. STZ diabetic rats were also found to exhibit significant decrease in glutathione level in liver as compare to control rats. Treatment with fresh juice and hydro alcoholic extract of fruits of *Embelica officinalis* produced significant increase in GSH level. The degree of increase GSH level was greater with hydro alcoholic extract as compared to fresh juice (Figs 1-3).

Table 1: Effect of fresh juice and hydroalcoholic extract of fruits of *Embelica officinalis* on BSL, pro-oxidant and antioxidant parameters in Streptozotocin induced diabetic rats

Parameters	CON (n=6)	DIC (n=6)	DIFJ (n=6)	DIHA (n=6)
MDA (nmoles/mg protein)	0.74 ± 0.11	1.56 ± 0.08*	0.57± 0.06*#	0.93 ± 0.1*#
SOD (units/min/mg protein)	1.20 ± 0.11	0.83 ± 0.07*	1.16 ± 0.13#	1.17 ± 0.09#
Catalase (units/min/mg protein)	1.88 ± 0.37	0.98 ± 0.24*	1.47 ± 0.17#	1.89 ± 0.31#
Glutathione (microgram/mg protein)	13.69 ± 1.04	4.82 ± 0.51*	7.86 ± 0.70*#	18.65 ± .85*#
Serum glucose (mg/dl)	99.1 ± 3.2	392.2 ±16.5*	147.2 ± 10.5#	106.2 ± 4.5#

CON – healthy control rats, DIC – STZ induced diabetic control rats, DIFJ - STZ induced diabetic rats treated with fresh juice of fruits of *E. officinalis* (5 ml/kg/p.o/day), DIHA - STZ induced diabetic rats treated with hydro alcoholic extracts of fruits of *E. officinalis* (100 mg/kg/p.o/day). Values are expressed as mean ± SEM, *- indicate significantly different from control ($p<0.05$), # - indicate significantly different from diabetic control ($p<0.05$)

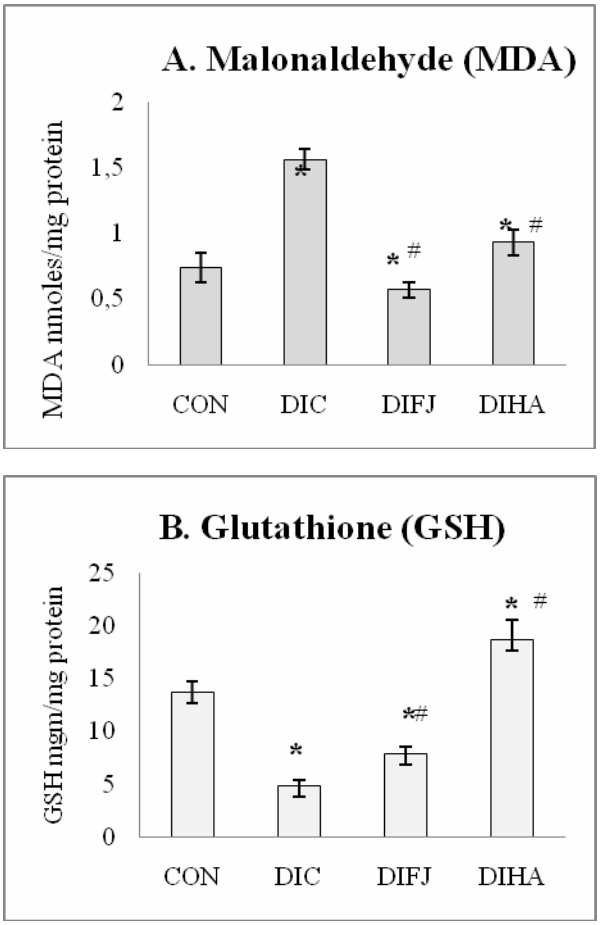
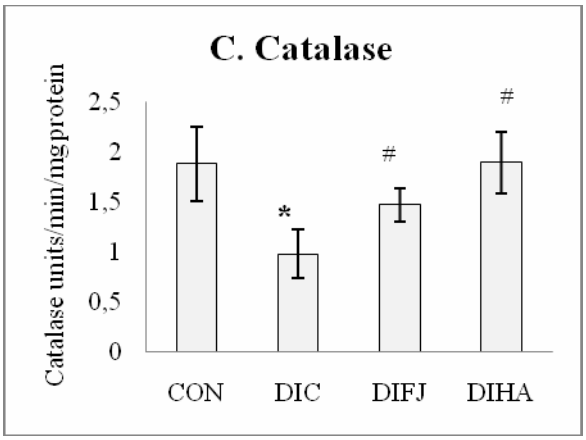


Fig 1: Effect on Malonaldehyde (A) and Glutathione (B) by chronic treatment with fresh juice and hydroalcoholic extract of *E. officinalis* in control and diabetic rats.

Each bar represents Mean + SEM of 6 animals. CON – healthy control rats, DIC – STZ induced diabetic control rats, DIFJ - STZ induced diabetic rats treated with fresh juice of fruits of E. officinalis (5 ml/kg/p.o/day), DIHA - STZ induced diabetic rats treated with hydro alcoholic extracts of fruits of E. officinalis (100 mg/kg/p.o/day). *- indicate significantly different from control ($p<0.05$), # - indicate significantly different from diabetic control ($p<0.05$)



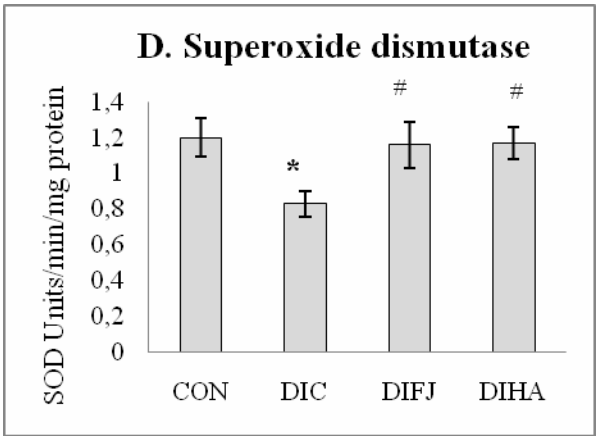


Fig 2 : Effect on Catalase (C) and superoxide dismutase (D) by chronic treatment with fresh juice and hydroalcoholic extract of *E. officinalis* in control and diabetic rats.

Each bar represents Mean + SEM of 6 animals. CON – healthy control rats, DIC – STZ induced diabetic control rats, DIFJ - STZ induced diabetic rats treated with fresh juice of fruits of E. officinalis (5 ml/kg/p.o/day), DIHA - STZ induced diabetic rats treated with hydro alcoholic extracts of fruits of E. officinalis (100 mg/kg/p.o/day). *- indicate significantly different from control ($p<0.05$), # - indicate significantly different from diabetic control ($p<0.05$)

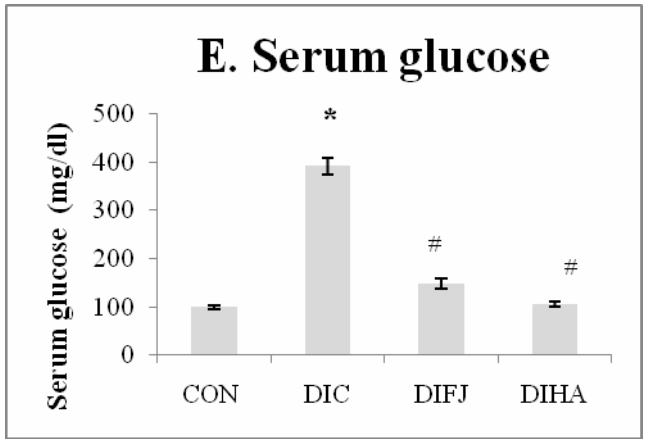


Fig 3 : Effect on Serum glucose by chronic treatment with fresh juice and hydroalcoholic extract of *E. officinalis* in control and diabetic rats.

Each bar represents Mean + SEM of 6 animals. CON – healthy control rats, DIC – STZ induced diabetic control rats, DIFJ - STZ induced diabetic rats treated with fresh juice of fruits of E. officinalis (5 ml/kg/p.o/day), DIHA - STZ induced diabetic rats treated with hydro alcoholic extracts of fruits of E. officinalis (100 mg/kg/p.o/day). *- indicate significantly different from control ($p<0.05$), # - indicate significantly different from diabetic control ($p<0.05$)

Discussion

Data of present study indicates antioxidant effect of fruits of *E. officinalis* in STZ induced type I diabetes in wistar rats. In our present study, IDDM rats showed significant increase in serum glucose, oxidative free radicals, MDA level and decrease in SOD, catalase, glutathione peroxidase levels in rat liver homogenates compared to normal rat. Treatment with fresh juice and hydro alcoholic extracts of *E. officinalis* significantly reduced in IDDM induced increase in serum glucose, MDA level in rat liver homogenates. Treatment with fresh juice reduced double fold the MDA levels compare to hydro alcoholic extract. Antioxidant enzymes SOD, catalase, glutathione peroxidase were decreased in IDDM rat liver indicating the dysfunction in antioxidant defensive system in diabetes. There was significant improvement in GSH level in diabetic rats treated with fresh juice and hydro alcoholic extracts, and degree of increase in GSH level was higher in hydro alcoholic extract compared to fresh juice. Treatment with fresh juice and hydro alcoholic extract increase in SOD and catalase levels in rat liver homogenate compared to diabetic control rats. In conclusion, our data suggested that fresh juice and hydro alcoholic extract of fruits of *Embelica officinalis* possesses potential antidiabetic and antioxidant as it decrease serum glucose and lipid peroxidation and enhanced antioxidant status in IDDM rats. Our study indicates the role of oxidative stress in diabetes.

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