ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Investigation into mechanism of action of anti-diabetic activity of *Emblica officinalis* on streptozotocin induced type I diabetic rat

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ABSTRACT

Objective of present investigation was to study anti-diabetic activity of fresh juice and hydro-alcoholic extract of fruits of *Emblica officinalis* Gaertn. (Euphorbiaceae) in streptozotocin(STZ) induce type 1 diabetic rats. Wistar rats were made diabetic with streptozotocin. Animals were divided into four groups namely non diabetic control, diabetic control, diabetic treated with fresh fruit juice and hydro-alcoholic extract of fruits of *E. officinalis*. Diabetic treated group received fresh juice and hydro-alcoholic extract of *E. officinali*, daily for four weeks. Control group received distilled water. STZ induced diabetic rats shows significant loss of body weight, polyuria and polydypsia. In STZ-diabetic rats, there was significant decrease in serum insulin levels and $AUC_{insulin}$ associated with significant increase in fasting BSL and $AUC_{glucose}$. Treatment with fresh juice and hydro-alcoholic extract significantly reduced elevated fastilng glucose and $AUC_{glucose}$ levels in type I diabetic rats. Treatment with fresh juice and hydro-alcoholic extract produce significant increase in serum insulin level and $AUC_{insulin}$ of diabetic rats compared to that of diabetic control. In conclusion, our data suggests, fresh juice and hydroalcoholic extract of *E. officinalis* fruits possesses potential anti-diabetic activitiy in STZ induce type 1 diabetic rat.

Key word: *Emblica officinalis,* streptozotocin, *diabetes*

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ISSN: 0975-8585

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, carbohydrates & proteins resulting from defects in insulin secretion, insulin action or both.[1] The chronic hyperglycemia is associated with long damage, dysfunction & failure of various organs especially eyes, kidneys, nerves, heart & blood vessels thus covering a wide range of heterogeneous disease. [1].

In 2000, according to World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of population.[2] Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double.[2] The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030.[2] The increase in incidence of diabetes in developing countries follows trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet.

The fruits of *Emblica officinalis* Gaertn. commonly known as amla or Indian gooseberry is known for its medicinal and therapeutic properties from ancient time in India and considered as a wonder fruit for health conscious population. It is extensively found throughout India and some other Asian countries. The fruits are widely consumed raw, cooked, or pickled. The fruits of plant form a major constituent of many potent Ayurveda preparations and these preparations are widely used for their preventive, curative, and health restorative properties. [5]

Amla contains highest amount of Vitamin C (ascorbic acid), low and high molecular weight tannins 30%, phyllembin (2.4%), phyllemblic acid (6.3%), gallic acid (1.32%), ellagic acid in natural form and cytokine like substances identified as Zeatin, Z riboside, Z nucleotide. [6] Amla fruit ash contains chromium, 2.5; zinc, 4; and copper, 3 ppm. Presence of chromium is of therapeutic value in diabetes. The fruit contains 482.14 units of superoxide dismutase/g fresh weight, and exhibited antisenescent activity. [6] Chromium, a trace element possesses significant antidiabetic activity in various experimental models of diabetic mellitus. Chromium compounds also improved deranged lipid metabolism of both type 1 and type 2 diabetic rats. [7] It has been reported that insulin derived with chromium is capable of reversing blood sugar, serum cholesterol and phospholipids levels to those of normal rats. [7]

IDDM patients may require injection of insulin for adequate control of glucose level. Further, in spite of anti-diabetic therapy patients may suffer from dyslipidaemia with increase in circulating triglycerides, very low-density lipoprotein (VLDL) and hence an increased morbidity and mortality due to diabetes induced cardiovascular complications. WHO has approved use of traditional medicines as a part of health programme. Hundreds of products are marketed in India as “natural” agents for lowering blood sugar and decreasing long term complications. According to WHO survey 80% of the population living in developing countries relies almost exclusively on traditional medicine for the primary health care needs. In

traditional medicine, the medicinal plants play a major role and constitute backbone of traditional medicine.

Synthetic hypoglycemic agents can produce serious side effects including hematological effects, coma and disturbances of liver and kidney. In addition, they are not suitable for use during pregnancy. [8] Compared to synthetic drugs, herbal preparations are frequently considered to be less toxic with fewer side effects. Therefore search for more effective and safer antihyperglycemic has become an area of current research.

In light of above facts, objective of present investigation was to investigaton into antidiabetic activitiy of fresh juice and hydro-alcoholic extract of fruits of *Emblica officinalis Gaertn* in STZ induced type 1 diabetc rats.

MATERIALS AND METHODS

Identification and collection of plant material

The fresh fruits of *Embllica officinalis* were purchased from Gaziabad (Madhypradesh) and fruits were identified and authenticated by Prof. O. P. Saxena, Head, Botany Department, Gujarat University, Ahmedabad, India.

Preperation 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 fresh juice with cotton clothe, thus juice obtained were used for treatment.

Hydroalcoholic extraction preparation

500 grams of the powder of shade dried fruits of *E. Officinalis* was extracted exhaustively in a round bottom flask with water and methanol mixture at 40-60^{o}C in proportion of 1:1 for 48 hours. The hydroalcoholic extracts thus obtained was filtered and solvent removed under vacuum.

Treatment protocol

Healthy Wistar rats of either sex weighing 150-200 gm were used for present study. The animals were housed in group of 3 rats per cage under well-controlled conditions of temperature (22 $\pm$ 2°C), humidity (55 ± 5%) and 12h/12h light-dark cycle. Animals had 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 the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Induction of type I Diabetes Mellitus

Diabetes was induced with streptozotocin (STZ) (Sigma, USA) 45 mg/kg dissolved in 0.9 % NaCl, administered as a single intravenous (i.v.) tail vein injection under light ether anesthesia. Control animals were injected with an equivalent volume of 0.9% NaCl. Animals were checked for extent of glucosuria 48 hours after injection of STZ using diastix (Bayer Diagnostics, India). Animals 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 streptozotocin.

The experimental animals were divided into four groups, six animals in each group and treatment is contiune for four weeks.

GP I : Normal control
GP II : Diabetic control
GP III : Diabetic rat treated with fruits of *E. officinalis* fresh juice (5 ml/kg, p.o., per day)
GP IV : Diabetic treated with fruits of *E. officinalis* hydro alcoholic extract (100 mg/kg/p.o./day)

Blood sample collection and serum analysis for diabetic parameter

At the end of 4 weeks treatment, blood samples were collected from rats in clean dry centrifuge tubes after 12 hours fast from retro orbital plexuses under light ether anesthesia and were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 5000 rpm for 20 min and stored at –20°C until analysis was carried out. Serum samples were analyzed for glucose using diagnostic kits (Span Diagnostics Ltd., India) calorimetrically using UV-Visible spectrophotometer (Shimadzu UV-1601, Japan). Serum insulin was estimated by radioimmunoassay (RIA) technique using kits obtained from Board of Radiation and Isotope Technology, Mumbai in gamma counter (BRIT).

Oral Glucose Tolerance Test (OGTT)

At the end of 4 weeks of treatment, oral glucose tolerance test was performed after an overnight fast. The animals were orally administered with 1.5 g/kg of glucose and blood samples were collected from tail vein under light ether anesthesia before i.e. 0 min and 30, 60 and 120 min after oral glucose administration. Samples were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 25 min and analyzed for glucose and insulin as explained earlier. Plotting glucose or insulin concentration versus time gives a curve showing rise and fall in glucose and insulin levels with time after an oral glucose

load. Comparison of such curves gives only a vague idea about alterations in insulin-mediated glucose disposal and insulin release in response to oral glucose load. Therefore, results were expressed as integrated area under curve (AUC) for glucose and insulin. This was calculated by applying trapezoid rule [AUC = $(C_1 + C_2)/2 \times (t_2 - t_1)$] and changes in glucose and insulin concentrations over 120 min during OGTT were expressed as $AUC_{glucose}$ (mg/dl.120min) and $AUC_{insulin}$ (µU/ml.120min) respectively.

***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 < 0.05 and highly significant at P < 0.001. Statistical analysis was performed using Sigma stat statistical software.

RESULTS

General features of animals during the study

Injection of streptozotocin (45mg/kg) into rats produced glucosuria (>2%) in all animals. No detectable glucose was present in urine of control animals.

Body weight, Food intake and Water intake

Intravenous injection of 45 mg/kg STZ in adult rats produced cardinal signs of type I diabetes i.e., loss of body weight (Fig 1), polyphagia (Fig 2A), and polydipsia (Fig 2B). Glucosuria (>2%) and polyuria observed in these animals persisted throughout period of four weeks. Chronic treatment with fresh juice (5ml/kg, p.o) and hydroalcoholic extract (100 mg/kg/p.o./day) did not prevent loss of body weight in STZ-diabetic rats. Treatment with fresh juice (5ml/ kg/p.o./day) could not reduce elevated food and water intake of diabetic rats but treatment with hydroalcoholic extract (100mg/kg/p.o./day) significantly reduced elevated food intake but not water intake of diabetic rats. There was no significant effect on food- and water intake of control rats. (Table1)

Table 1:- Effect of *E. officinalis* fresh juice and hydroalcoholic extract treatment on general features of control and diabetic rats.

PARAMETERS	CON (n=6)	DIC (n=6)	DIFJ (n=6)	DIHA (n=6)
Body weight After treatment (gms)	245.83 ± 6.28	219.23 * ± 5.42	223.25* ± 5.85	222.63* ± 5.6
Food intake (gm/animal/day)	30.14 ± 7.12	46.53* ± 7.35	41.97* ± 3.65	35.58*# ± 4.82
Water intake (ml/animal/day)	23.33 ± 6.55	42.08* ± 9.46	38.96* ± 7.0 6	37.60* ± 8.21

ISSN: 0975-8585

Values are expressed as Mean ± S.E.M
*- significantly different from control ($p < 0.05$)
- significantly different from diabetic control ($p < 0.05$)
CON - control animals, DIC - diabetic control animals, DIM - diabetic animals treated with fresh fruit juice of *E. officinalis* (5ml/ kg/p.o./day), DIHA - diabetic animals treated with Hydro Alcoholic extract of *E. officinalis* (100mg/kg/p.o./day).

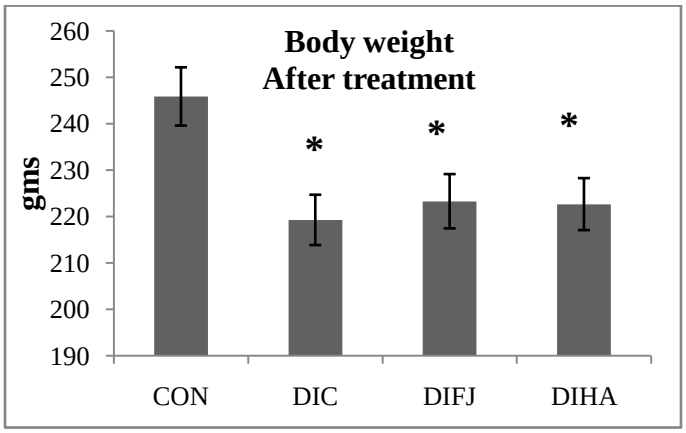
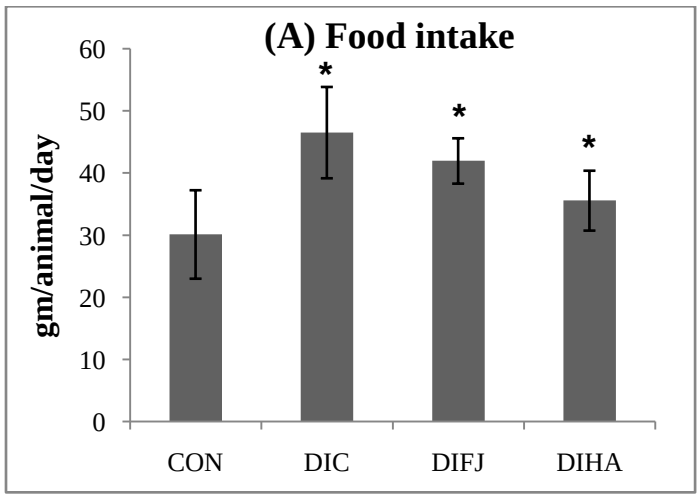


Fig 1. Effect on body weight after treatment of four weeks with fresh juice and hydroalcoholic extract in control and diabetic rats.

Each bar represents Mean ± SEM of 6 animals. *- significantly different from control ($p < 0.05$)
CON - control animals, DIC - diabetic control animals, DIM - diabetic animals treated with fresh fruit juice of *E. officinalis* (5ml/ kg/p.o./day), DIHA - diabetic animals treated with Hydro Alcoholic extract of *E. officinalis* (100mg/kg/p.o./day).



ISSN: 0975-8585

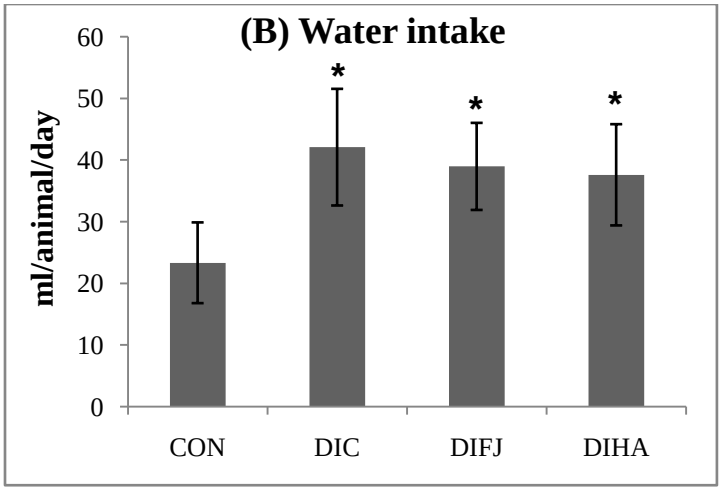


Fig 2. Effect on Food intake (A) and Water intake (B) by chronic treatment with fresh juice and hydroalcoholic extract in control and diabetic rats.

Each bar represents Mean ± SEM of 6 animals. CON – non-diabetic control animals. DIC – diabetic animals. DIFH – diabetic animals treated with fresh juice (5ml/ kg/p.o./day). DIHA – diabetic animals treated with hydroalcoholic extract. (100mg/kg/p.o./day).

***** - Significantly different from control animals. ($P<0.05$)

BIOCHEMICAL PARAMETERS

Serum Glucose and Insulin

STZ-diabetic rats were found to exhibit significant hyperglycemia (Fig 3A) with a corresponding hypoinsulinaemia (Fig 3B) as compared to control rats. Treatment with fresh juice (5ml/ kg/p.o./day) and hydroalcoholic extract (100 mg/kg/p.o./day) produced significant decrease in elevated serum glucose levels (Fig 3A). Decrease in serum insulin levels in diabetic rats was significantly prevented by the treatment with fresh juice and hydroalcoholic extract (100mg/kg/p.o./day) (Fig 3B) (Table 2).

Oral Glucose Tolerance Test (OGTT)

Administration of glucose (1.5 g/kg, p.o.) did not produce any significant change in the serum glucose levels of control treated group animals throughout 120 min (fig 4A). Following oral glucose administration, serum glucose in diabetic groups increased. Serum glucose levels of diabetic controls remained high and there was no decline in the $AUC_{glucose}$ levels (fig 4A).

$AUC_{glucose}$ of diabetic control group was significantly higher compared to that of control. Treatment with fresh juice and hydroalcoholic extract produced a significant decrease in $AUC_{glucose}$ of diabetic rats compared to that of diabetic control (fig 4A) (table 2).

$AUC_{insulin}$ of diabetic control was significantly lower as compared to that of control group. Treatment with fresh juice and hydroalcoholic extract did not produce any significant change

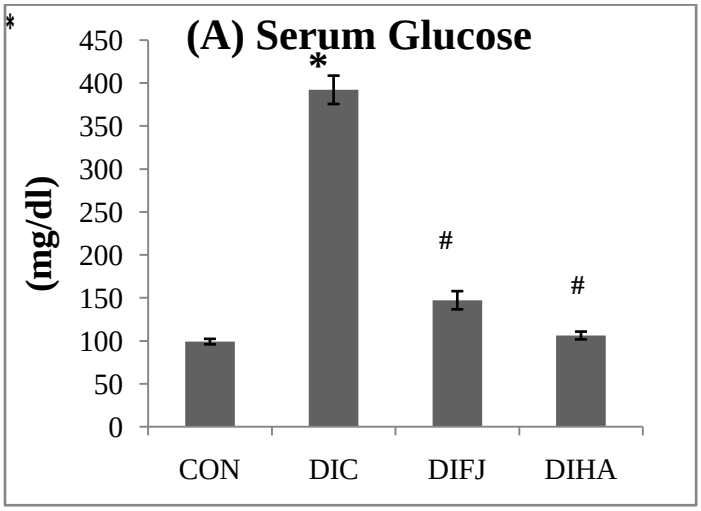
ISSN: 0975-8585

but produce a slight improvement in $AUC_{insulin}$ of diabetic rats as compared to that of diabetic control.

Table 2:- Effect of *E. officinalis* fresh juice and hydro alcoholic extract treatment on biochemical parameters of control and diabetic rats.

PARAMETER	CON (n=6)	DIC (n=6)	DIFJ (n=6)	DIHA (n=6)
Serum glucose (mg/dl)	99.09 ± 3.20	392.22* ± 16.52	147.25# ± 10.57	106.22# ± 4.52
Serum insulin (µU/ml)	18.75 ± 2.01	12.33* ± 1.33	18.6# ± 0.92	17.83# ± 1.62
$AUC_{glucose}$ (mg/dl.min)×10^3	14.3 ± 0.67	49.56* ± 2.38	38.26*# ± 1.68	37.24*# ± 2.55
$AUC_{insulin}$ (mg/dl.min)×10^3	6.45 ± 0.48	2.32* ± 0.16	2.86* ± 0.15	3.0* ± 0.2

Values are expressed as Mean ± S.E.M
*- significantly different from control ($p < 0.05$)
- significantly different from diabetic control ($p < 0.05$)
CON - control animals, DIC - diabetic control animals, DIM - diabetic animals treated with fresh fruit juice of *E. officinalis* (5ml/ kg/p.o./day), DIHA - diabetic animals treated with Hydro Alcoholic extract of *E. officinalis* (100mg/kg/p.o./day).



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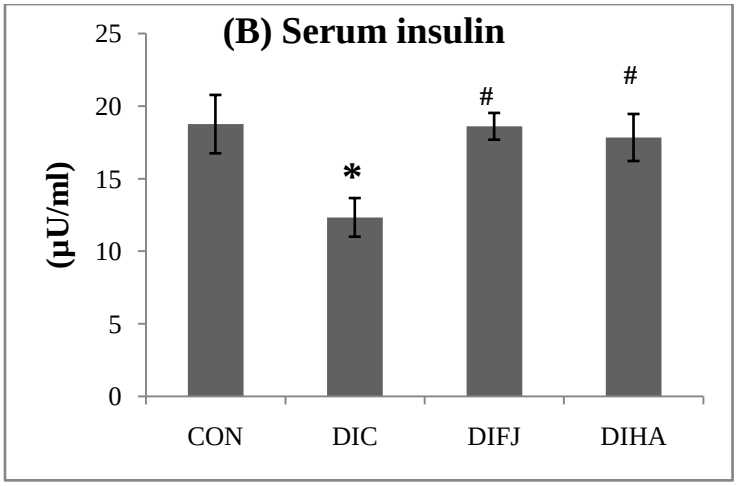
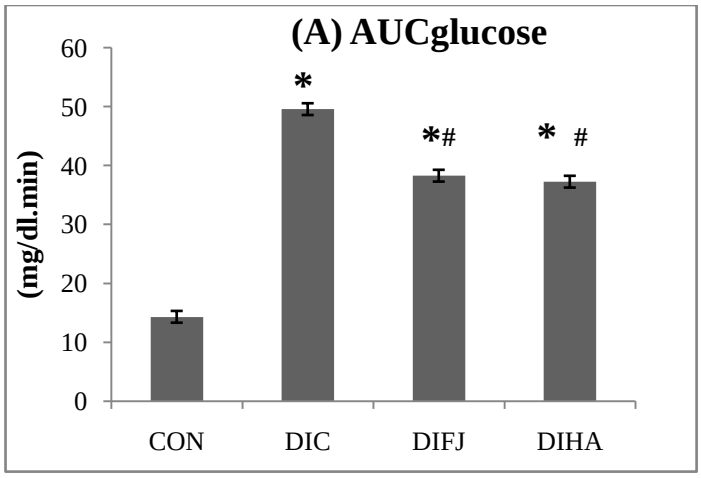


Fig 3. Effect on Serum Glucose Levels (A) and Serum Insulin Levels (B) by chronic treatment with fresh juice and hydroalcoholic extract of *E. officinalis* in control and diabetic rats.

Each bar represents Mean ± SEM of 6 animals. CON – non-diabetic control animals. DIC – diabetic animals. DIFH – diabetic animals treated with fresh juice (5ml/ kg/p.o./day). DIHA – diabetic animals treated with hydroalcoholic extract. (100mg/kg/p.o./day).

∗ - Significantly different from control animals. (P<0.05)

- Significantly different from diabetic group. (P <0.05)



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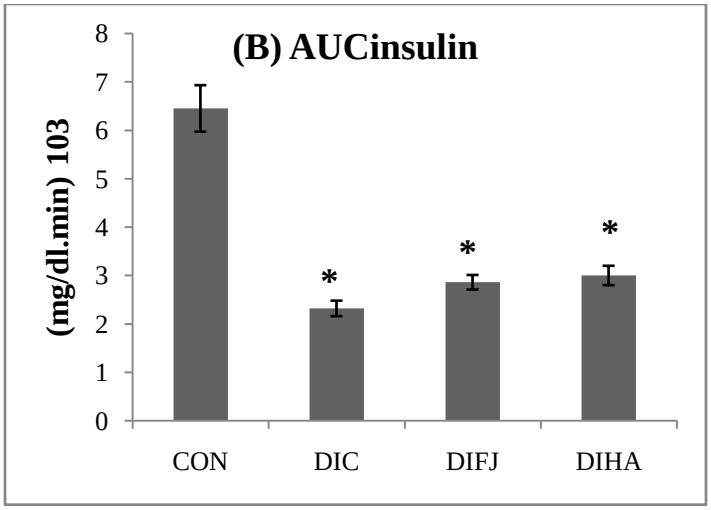

Fig 4. Effect on AUCglucose (A) and AUCinsulin (B) by chronic treatment with fresh juice and hydroalcoholic extract of *E. officinalis* in control and diabetic rats.

Each bar represents Mean ± SEM of 6 animals. CON – non-diabetic control animals. DIC – diabetic animals. DIFH – diabetic animals treated with fresh juice (5ml/ kg/p.o./day). DIHA – diabetic animals treated with hydroalcoholic extract. (100mg/kg/p.o./day).

∗ - Significantly different from control animals. (P<0.05)

- Significantly different from diabetic group. (P <0.05)

DISCUSSION

Data of present study indicates anti-diabetic effect of *Emblica officinalis* in STZ induced type 1 diabetes in wistar rats. Streptozotocin (STZ), a β-cytotoxin, induces 'chemical diabetes' in a wide variety of animal species including rat by selectively damaging insulin-secreting β-cells of pancreas. Intravenous injection of STZ produces fragmentation of DNA of β-cells of pancreas which stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of β-cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia. [9] A significant loss of body weight was found in diabetic rats over the period of four weeks. Chronic treatment with fresh juice (5ml/ kg/p.o./day) and hydroalcoholic extract (100mg/kg/p.o./day) could not prevent loss of body weight in STZ-diabetic rats. Treatment also did not alter the normal gain in body weight of control rats. The loss of body weight of diabetic rats as compared to that of control rats could be due to emaciation of skeletal muscle, dehydration and catabolism of fats and proteins. [10-12] The other cardinal signs of STZ diabetes viz., polyphagia, polyuria and polydipsia were also observed in our study and they are consistent with those reported earlier. [13-14] Treatment with fresh juice could not reduce elevated food- and water- intake of diabetic rats. There was significant reduction in elevated food intake but not water intake in diabetic rats treated with hydroalcoholic extract.

STZ type I diabetes produced a significant increase in glucose levels associated with decrease in insulin levels. Treatment with fresh juice and hydroalcoholic extract of *E. officinalis*

showed significant decrease in fasting serum glucose level which was near to healthy control and increase in insulin levels as compared to diabetic rats.

At the end of treatment schedule animals were subjected to Oral Glucose Tolerance Test (OGTT) which directly measures action of endogenous insulin in response to a glucose stimulus. [15] However, this method does not allow a separate evaluation of β islet cells and peripheral insulin sensitive tissues. In present investigation, there was a significant increase in $AUC_{glucose}$ along with a significant decrease in $AUC_{insulin}$ in type I diabetic rats as compared to non diabetic control rats. Treatment with fresh juice and hydroalcoholic extract showed significant decrease in $AUC_{glucose}$ and slightly increase $AUC_{insulin}$ but it is not significant.

In conclusion, our data suggest the fresh juice and hydroalcoholic extract of fruits of *E. officinalis* possess potential antidiabetic activity as it lowers serum glucose level and significantly increases glucose tolerance in STZ induced type 1 diabetic rat.
Probable mechanism and active constituents of *E. officinalis* responsible for antidiabetic activity reqiures further to be investigated.

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