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Antidiabetic and antioxidant potential of *Emblica officinalis* Gaertn. leaves extract in streptozotocin-induced type-2 diabetes mellitus (T2DM) rats

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ABSTRACT

Ethnopharmacological relevance: In traditional Indian medicine, all parts of *Emblica officinalis* Gaertn plant including the fruit, seed, leaves, root, bark and flowers are used in various herbal preparations for the treatment of diabetes mellitus, chronic diarrhea, anti-inflammatory and antipyretic.
Aim of the study: To evaluate the hypoglycemic and antioxidants effects of the hydro-methanolic (20:80) extract of leaves of *Emblica officinalis* Gaertn. (HMELEO) in streptozotocin induced diabetic rats.
Material and methods: The hypoglycemic effect was measured by blood glucose and plasma insulin level. The oxidative stress was measured in liver and kidney by level of antioxidant markers *i.e.* lipid peroxidation (LPO), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT), and the biochemical parameters, i.e. blood serum levels of creatinine, urea, serum glutamic pyruvic transaminases (SGPT), serum glutamic oxaloacetic transaminases (SGOT), alkaline phosphatase (ALP), total cholesterol and triglyceride levels were the salient features observed in diabetic control and treated rats.
Results: Oral administration of the HMELEO at a concentration of 100, 200, 300 and 400 mg/kg b.w. daily for 45 days showed a significant ($P < 0.05$) decrease in fasting blood glucose and increase insulin level as compared with the diabetic rats. Also it significantly ($P < 0.05$) reduced all biochemical parameters (serum creatinine, serum urea, SGOT, SGPT and lipid profile). The treatment also resulted in a significant ($P < 0.05$) increase in reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase, and decrease LPO level in the liver and kidney of diabetic rats.
Conclusion: The results clearly suggest that the hydro methanolic extract of leaves of *Emblica officials* Gaertn. treated group may effectively normalize the impaired antioxidant status in streptozotocin induced diabetes at dose dependent manner than the glibenclamide-treated groups. The extract exerted rapid protective effects against lipid peroxidation by scavenging of free radicals and reducing the risk of diabetic complications.

1. Introduction

Diabetes is a deadly disease that affected an estimated 285 million people worldwide in 2010 and the number is increasing in rural and poor populations throughout the world and is projected to become one of the world's main disablers and killers within the next 25 years (Shaw et al., 2010). The developed countries such as India, China, and the U.S. are presently the countries with the leading number of diabetics. Furthermore, seven percent of the residents of the United States are diabetic. Though it is a non-communicable disease, but is considered to be one of the five leading causes of death world-wide (Chakraborty and Das, 2010). It is a complex metabolic disorder of the endocrine system with dynamic expression of pathological disequilibria, resulting in various micro and macro vascular complications. It is characterized by high blood glucose levels (hyperglycemia) due to the inability of the body's cells to utilize glucose properly (West, 2000). The increased blood glucose levels in diabetes produce superoxide anions, which generate hydroxyl radicals via Haber–Weiss reaction, resulting in peroxidation of membrane lipids and protein glycation. This leads to oxidative damage of cell membranes. These radicals further damage other important biomolecules including carbohydrates, proteins and deoxyribonucleic acid (DNA) (Baynes, 1991).

In diabetes, oxidative stress has been found mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defenses (Oberley, 1988). The endogenous antioxidant enzymes (e.g. SOD, CAT, GSH and GPx) are responsible for the detoxification of deleterious oxygen radicals

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(Jacob, 1995). Antioxidants thus play an important role to protect the human body against damage caused by reactive oxygen species (Baynes, 1991). Hence, compounds with both hypoglycemic and antioxidative properties would be useful antidiabetic agents (Baynes, 1995).

Experimental diabetes mellitus has been induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. The second method for creating diabetes in animals is injecting drugs such as alloxan or Streptozotocin (STZ). Oxidative stress is increased in experimental models of streptozotocin induced diabetes mellitus. The cytotoxic action of streptozotocin selectively destroys β-cells of pancreas by generating excess ROS and carbonium ion (CH^{3+}) leading to DNA breaks by alkylating DNA bases causing oxidative damage (Szkudelski, 2001).

Now a days, scientists and researchers are very much trying on research of natural plant products all over the World and a large number of substantiation have shown the immense potential of medicinal plants used traditionally (Habib et al., 2005). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes. The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest (Kameswara and Appa, 2001). Therefore considerable focus has been given to an intensive search for novel type of antioxidants from numerous plant materials for management of diabetes without any side effects.

Emblica officinalis Gaertn. belongs to the family Euphorbiaceae. In traditional Indian medicine, all parts of the plant including the fruit, seed, leaves, root, bark and flowers are used in various herbal preparations. The plant is used in many forms. One of the most popular is as a decoction and infusion of leaves and seeds. Traditional use of *Emblica officinalis* Gaertn. leaves is against cold, in anemia, dysentery, fever, gravel, sores (*agya ghao*, *rokoc ghao*) (Dey, 1896). Decoctions of the leaves are used in the treatment of diabetes mellitus (Treadway, 1994) and chemical-free bactericidal mouthwash in the treatment of aphthae (Nadkarni and Nadkarni, 1999). An infusion of the leaves with fenugreek seed is given for chronic diarrhea (Jayaweera, 1980). Green fresh leaves combined with curds have carminative and stomachic effect (Nadkarni and Nadkarni, 1999). Leaves have been used for anti-inflammatory and antipyretic treatments (Burkill, 1966). The milky juice of the leaves is a good application to sores (Treadway, 1994). Infusion of the leaves is applied to sore eyes (Drury, 1873). *Emblica officinalis* Gaertn. leaves are rich in tannins, amlaic acid, astragalin, ellagic acid, gallo-tannin, kaempferol, kaempferol-3-o-glucoside, phyllantidine, phyllantine and rutin (Thakur et al., 1989; Duke, 1992).

Despite a long traditional use of *Emblica officinalis* Gaertn. leaves in diabetes, no systematic phytochemical and pharmacological work has been carried out on this potential plant. So the aim of the present investigation is to find out *Emblica officinalis* Gaertn leaves as an alternative natural remedy for the treatment of diabetes.

2. Material and methods

2.1. Chemical used

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. Glibenclamide (standard drug) was a gift sample from Wokhardt Pvt. Ltd. All other commercial reagents used were of analytical grade.

2.2. Animals

Male albino wistar rats aged 7–8 weeks (200–250 g) were used. Animals were kept in animal house at an ambient temperature of 25–30 °C and 45–55% relative humidity with a 12 h each of dark and light cycle. Animals were fed pellet diet and water ad libitum. The experimental protocol has been approved by the institutional animal ethics committee (Protocol no. MMCP/IEC/10/11).

2.3. Plant Materials

Leaves of *Emblica officinalis* Gaertn. were collected in the month of June from national park, Yamunanagar and authenticated from NISCAIR, (Ref. no. NISCAIR/RHMD/CONSULT/2009-2010/1336/138), New Delhi. Leaves were air dried, and pulverized (coarse power).

2.4. Preparation of the Hydro-methanolic extract

The coarse powder (250 g) was extracted with 500 ml of hydro-methanol (20:80) using a soxhlet extractor for 7 h at a temperature (64 °C) not exceeding the boiling point of the solvents. The extract was filtrated using whatman filter paper (no. 1) and then concentrated at 40 °C using a rotary evaporator. The residue (21 g) obtained was stored in freezer at −80 °C until further tests.

2.5. Preliminary phytochemicals studies

The extract was subjected to various phytochemicals tests to determine the active constituents present in the crude hydro-methanolic leaves extracts of *Emblica officinalis* Gaertn. (Okerulu and Ani, 2001).

2.6. Acute toxicity study

Acute oral toxicity study was performed as per OECD (Organization of Economic Cooperation and Development) 423 guideline (Acute toxic class method). Healthy male albino wistar rats were randomly divided into 6 groups with 6 animals in each group. The animals were kept fasting overnight providing only water, after which the hydro-methanolic extract of *Emblica officinalis* Gaertn. leaves was administered orally with increasing doses (10, 50, 100, 500, 1000 and 2000 mg/kg) by intragastric tube to determine the safe doses by up and down staircase method (Ghosh, 1984). The animals were observed continuously for 1 h, then frequently for 4 h and later at the end of 24 h for general behavioral, neurological and autonomic profile. Further, one group was administered high dose of *Emblica officinalis* Gaertn. leaves extract orally once daily for 15 days and observed for any lethality and death (Turner, 1965).

2.7. Induction of experimental diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (50 mg/kg b.w.) in 0.1 M citrate buffer (pH 4.5) (Hemalatha et al., 2004). The animals were allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia (Balasubramaian et al., 2004). On the third day of STZ-injection, the rats were fasted for 6 h and blood was withdrawn by retro orbital puncture under light ether anesthesia. Rats with moderate diabetes having hyperglycemia (that is, with blood glucose of 250–400 mg/dl) were taken for the experiment (Burcelin et al., 1995).

2.8. Animal grouping

In the experiment, a total of 42 rats (36 diabetic surviving rats and six normal rats) were used. The rats were divided into seven groups of six rats each: group-I, normal control (untreated) rats;

group-II, diabetic control rats; group-III, diabetic rats given glibenclamide (1 mg/kg body weight); group-IV, diabetic rats given *Emblica officinalis* Gaertn. leaves extract (100 mg/kg body weight), group-V, diabetic rats given *Emblica officinalis* Gaertn. leaves extract (200 mg/kg body weight); group-VI, diabetic rats given *Emblica officinalis* Gaertn. leaves extract (300 mg/kg body weight); group-VII, diabetic rats given *Emblica officinalis* Gaertn. leaves extract (400 mg/kg body weight). The experimentation was carried out for 45 days, with oral administration of *Emblica officinalis* Gaertn. leaves extract.

2.9. Biochemical analysis

Rats were fasted overnight and the blood was withdrawn by retro orbital puncture under light ether anesthesia on the 1st, 22nd and 45th day post induction to determine blood glucose and plasma insulin level. The change in body weight was observed throughout treatment period in the experimental animals.

At the end of 45 days, the animals were deprived of food overnight and sacrificed by cervical decapitation for biochemical parameters (i.e. hemoglobin, glycosylated Hb, total protein, serum creatinine, serum urea, SGOT, SGPT, alkaline phosphate and lipid profile) and antioxidant enzyme (SOD, CAT, GSH, GPx, LPO) estimation. Blood was collected from the heart in two different tubes, i.e. one with anticoagulant for plasma, and another without anticoagulant for serum separation. Serum was separated by centrifugation 3500 × *g* at 25 °C for 10 min. Fasting blood glucose was estimated by O-toluidine method (Sasaki et al., 1972). Plasma insulin level was assayed by the radio-immunoassay method. All other biochemical tests were carried out in our lab by using commercial kits obtained from Erba diagnostic Mannheim Gmbh, Germany.

2.10. Oral glucose tolerance test

The rats were divided into four groups of 6 animals ($n=6$) each. Group I served as control and received distilled water. Group II served as diabetic control and received distilled water. Group III served as positive control, received glibenclamide (1 mg/kg b.w.). Group IV received HMELEO 400 gm/kg orally. The rats were fasted for 18 h and the test performed by oral administration of glucose (2 g/kg) to diabetic and normal rats 30 min after dosing. Blood samples were collected from the retro-orbital plexus of the eye (under light ether anesthesia) immediately (0 h), 30, 60, 90, and 120 min after the glucose administration and the blood glucose levels were estimated.

2.11. Preparation of liver and kidney homogenate

The liver and kidney were carefully removed, weighed and washed in ice-cold saline to remove the blood. Then both were sliced separately into pieces and homogenized with buffer containing 0.25 M sucrose and 0.1 M TrisHCl buffer (pH 7.4). The homogenate was centrifuged at 3000 × *g* for 10 min at 0 °C in cold centrifuge. The supernatant was separated and used for various antioxidant enzyme estimations.

2.12. Assay of antioxidant enzyme

The levels of lipid peroxidation (LPO) in tissues were estimated by the method of Okhawa et al. (1979). Superoxide dismutase (SOD) was assayed by the method of Kakkar et al. (1984). The activity of catalase (CAT) was determined by the method of Sinha (1972). Glutathione peroxidase (GPx) was estimated by the method of Rotruck et al. (1973). Reduced glutathione (GSH) was estimated by the method of Ellman (1959).

2.13. Statistical analysis

All the grouped data was statistically evaluated. Hypothesis testing method included one-way analysis of various (ANOVA) followed by Dunnette's comparison tests. *P*-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SEM for 6 animals in each group.

3. Results

3.1. Preliminary phytochemistry of the plant extract

Preliminary phytochemical analysis revealed that the plant possessed phytoconstituents tannins, phenolic compounds, and flavonoids.

3.2. Acute toxicity studies

Animals showed good tolerance to testing single doses of hydro-methanolic extract of *Emblica officinalis* Gaertn. leaves in doses as high as 2 g/kg that were found to be non-lethal. Highest dose of extract did not show any noticeable signs of toxicity and mortality after once daily administration orally for 15 days. So, the extract is safe for long term administration.

3.3. Effect on body weight

Decrease in body weight due to derangement of metabolic pathways is a common feature in diabetes (Al-Shamaony et al., 1994). But in the present study hydro-methanolic extract of *Emblica officinalis* Gaertn. leaves, to diabetic rats (Groups V and VII) increased body weight significantly ($p<0.05$) which was comparable to increase in the body weight of normal controls (Fig. 1).

3.4. Effect on blood glucose and plasma insulin level

Fasting blood glucose levels in the normal controls rats remained unchanged during the course of the experiment. In diabetic groups, level of fasting blood glucose was significantly ($p<0.05$) higher and the plasma insulin level was significantly decreased as compared to normal control group. On the other hand, administration of hydro-methanolic leaves extract of *Emblica officinalis* Gaertn. for 45 days was found to lower the blood glucose and increase the insulin level

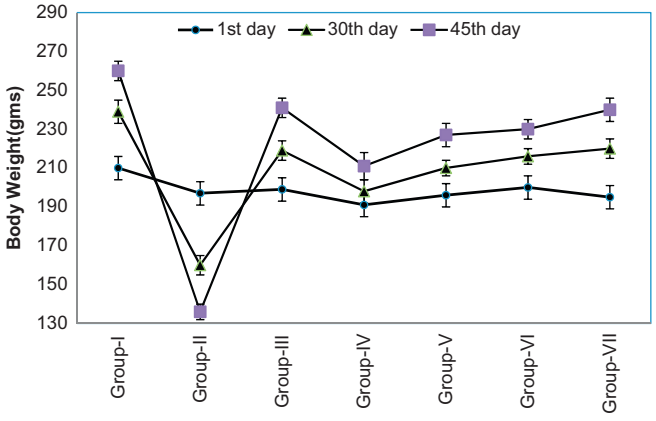

Fig. 1. Effect of hydro-methanolic extract of leaves of *Emblica officinalis* Gaertn. on body weight.

Table 1
Effect of HMELEO on blood sugar and plasma insulin level in streptozotocin induced diabetic rats.

Groups	Blood sugar (mg/dL)			Plasma insulin (μU/ml)		
	Initial	On 22nd day	On 45th day	Initial*	On 22nd day	On 45th day
Groups-I	71.27 ± 3.0	73.61 ± 2.5	74.43 ± 2.6	15.33 ± 0.81	16.07 ± 0.88	15.90 ± 0.56
Groups-II	298.6 ± 2.28	314.83 ± 5.7	340.21 ± 7.3	06.07 ± 0.78	04.97 ± 0.93	03.72 ± 0.73
Groups-III	282.61 ± 2.7	167.14 ± 3.4*	79.33 ± 2.5*	05.87 ± 0.83	10.45 ± 0.89*	14.70 ± 0.81*
Groups-IV	283.21 ± 3.9	249.27 ± 4.3	202.25 ± 5.7*	06.33 ± 0.95	07.11 ± 1.94	07.93 ± 0.81*
Groups-V	290.61 ± 3.2	224.31 ± 4.8*	163.71 ± 4.0*	07.06 ± 1.15	08.87 ± 2.09*a	09.04 ± 1.71*
Groups-VI	287.67 ± 2.9	207.23 ± 4.3*a	137.42 ± 3.1*a	07.52 ± 1.12	10.34 ± 1.56*a	11.78 ± 1.30*a
Groups-VII	279.17 ± 2.6	179.21 ± 4.1*a	103.56 ± 3.4*a	06.87 ± 1.17	09.71 ± 0.90a	12.89 ± 1.10*a

Data are expressed as mean ± SEM, $n=6$, initial means 3rd day (after 72 h) of stz injection.

* $p < 0.05$ when experimental groups were compared with diabetic control.

a $p < 0.05$ when experimental groups were compared with glibenclamide treated group.

Table 2
Effect of HMELEO on biochemical parameter in streptozotocin induced diabetic rats.

Parameter/Groups	Groups-I	Groups-II	Groups-III	Groups-IV	Groups-V	Groups-VI	Groups-VII
Hemoglobin (mg/dL)	13.43 ± 2.1	8.38 ± 1.2	11.92 ± 2.3	09.56 ± 1.1*	10.19 ± 1.9*a	11.97 ± 1.7*a	12.46 ± 1.6*a
Glycosylated Hb (Hb%)	6.85 ± 0.93	14.45 ± 1.1	7.21 ± 0.87	12.70 ± 0.9	11.15 ± 0.90*	9.70 ± 0.83*a	8.34 ± 0.89*a
Serum creatinine (mg/dL)	0.92 ± 0.1	2.10 ± 0.3	0.99 ± 0.3*	1.72 ± 0.2*	1.25 ± 0.4*	1.10 ± 0.3*a	0.99 ± 0.4*a
Serum urea (mg/dL)	32.50 ± 2.1	84.93 ± 2.0	36.97 ± 1.5*	78.30 ± 2.3	67.08 ± 2.1*	56.33 ± 1.7*a	42.15 ± 2.5*a
Total protein (g/dL)	7.6 ± 1.3	5.1 ± 1.4	7.2 ± 1.5*	6.0 ± 1.1*	6.5 ± 1.3*a	6.9 ± 1.0*a	7.4 ± 1.5*a
Alkaline phosphate (IU/L)	119.4 ± 6.3	291.6 ± 5.7	128.7 ± 6.1*	242.3 ± 6.4	191.2 ± 5.8*	169.9 ± 5.9*a	135.6 ± 7.0*a
SGOT (IU/L)	19.14 ± 3.0	45.27 ± 3.3	20.29 ± 3.9*	42.63 ± 3.8	34.56 ± 3.5*	27.78 ± 4.2*a	21.32 ± 3.9*a
SGPT (IU/L)	25.21 ± 4.9	57.63 ± 4.8	28.07 ± 5.3*	51.48 ± 5.7	42.34 ± 5.1*	33.87 ± 5.0*a	26.34 ± 5.2*a
Total cholesterol (mg/dL)	128.9 ± 5.7	259.8 ± 6.1	154.4 ± 6.6*	242.8 ± 5.9	203.07 ± 6.3*	170.3 ± 4.4*a	142.7 ± 4.9*a
Triglycerides (mg/dL)	81.1 ± 2.9	179.7 ± 3.2	90.3 ± 2.5*	163.1 ± 2.8	133.6 ± 3.3*	112.4 ± 3.5*a	97.34 ± 3.4*a
HDL (mg/dL)	51.30 ± 3.4	17.61 ± 3.2	48.35 ± 2.9*	22.72 ± 3.1	30.54 ± 3.2*	39.43 ± 3.3*a	48.81 ± 4.1*a

Data are expressed as mean ± SEM, $n=6$.

* $p < 0.05$ when experimental groups were compared with diabetic control.

a $p < 0.05$ when experimental groups were compared with glibenclamide treated group.

Table 3
Effect of hydro-methanolic extract of *Emblica officinalis* Gaertn leaves on OGTT in stz-induced diabetic rats.

Group treatment ($n=6$)	Fasting blood glucose level (mg/dL) at (h)				
	0 h	0.5 h	1 h	1.5 h	2 h
Group-I	97.25 ± 0.64	125.30 ± 1.67	134.59 ± 1.42	142.67 ± 1.78	150.31 ± 1.59
Group-II	250.36 ± 1.47	270.63 ± 0.95	282.47 ± 1.36	296.62 ± 1.10	309.56 ± 1.34
Group-III	253.20 ± 1.29	267.83 ± 1.81*	276.17 ± 1.01*	289.31 ± 1.65*	268.61 ± 1.38*
Group-IV	255.47 ± 1.64	266.98 ± 1.37*	279.37 ± 1.49*	298.76 ± 1.37*	270.17 ± 1.80*

* $p < 0.05$, values are mean ± SEM, $n=6$.

significantly ($p < 0.05$) in a dose dependent manner when compared with positive control groups (Table 1).

3.5. Effect on biochemical parameter

In order to examine the effect of *Emblica officinalis* Gaertn. supplementation on the regulation of biochemical parameters i.e. SGOT, SGPT and alkaline phosphate of the diabetic rats were to evaluate the hepatic function, while creatinine and urea concentration were studied to assess the renal function. STZ induced significant ($p < 0.05$) elevations in SGOT, SGPT, alkaline phosphate, serum creatinine and serum urea level when compared to control group. However, administration of hydro-methanolic extract of *Emblica officinalis* Gaertn. leaves for 45 days significantly ($p < 0.05$) reduced SGOT, SGPT and ALP level in diabetic groups at dose dependant manner. On the other hand, treatment with different doses of leaves extract of *Emblica officinalis* Gaertn. significantly ($p < 0.05$) reduced serum creatinine and serum urea level and increase total protein when compared to those of diabetic groups (Table 2).

3.6. Effect of hydro-methanolic extract of Emblica officinalis Gaertn leaves on oral glucose tolerance test (OGTT)

The results from the study clearly indicated that the hydro-methanolic extract of *emblica officinalis* Gaertn leaves (400 mg/kg) and glibenclamide (1 mg/kg) reduced the blood glucose level (hyperglycemia due to glucose load 2 g/kg p.o.) significantly ($p < 0.05$) after 120 min of oral administration, when compared to diabetic control (Table 3).

3.7. Antioxidant status in liver and kidney tissue

Oxidative stress assessment was performed by recording the activities of anti-oxidative enzymes i.e. catalase (CAT), Glutathione peroxidase (GPx), superoxide dismutase (SOD), reduced glutathione (GSH) and lipid peroxidation. The diabetic rats showed significant ($p < 0.05$) increase in TBARS along with significantly decreased level of antioxidant enzymes i.e. CAT, GPx, GSH and SOD in hepatic and renal tissues. Treatment with HMELEO significantly ($p < 0.05$) increased CAT, GPx, GSH and SOD in hepatic and renal tissues in

Table 4
Effect of HMELEO treatment for 45 days on superoxide dismutase, catalase, glutathione peroxidase, glutathione and lipid peroxidation in liver of control and experimental groups of rats.

Groups	Liver				
	SOD (units/mg protein)	CAT (μmol/min/mg protein)	GPx (μmol/min/mg protein)	GSH (mM/100 g tissue)	TBARS (μmol/100 g tissue)
Group-I	8.31 ± 1.29	86.43 ± 2.27	10.32 ± 0.91	52.11 ± 1.70	0.97 ± 0.27
Group-II	3.77 ± 0.17[a]	30.29 ± 2.09[a]	5.44 ± 0.89[a]	23.37 ± 2.10[a]	2.03 ± 0.19[a]
Group-III	6.23 ± 0.25*	78.32 ± 3.04*	10.01 ± 1.24*	47.23 ± 1.81*	0.90 ± 0.22*
Group-IV	4.73 ± 0.19	43.24 ± 2.21*	07.03 ± 1.02*	33.27 ± 2.09	1.86 ± 0.57
Group-V	5.80 ± 0.20*	57.67 ± 3.49*	08.50 ± 1.05*	39.89 ± 2.11*	1.50 ± 0.51*
Group-VI	6.41 ± 0.18*	69.14 ± 3.05*	09.70 ± 1.04*	45.36 ± 2.83*	1.17 ± 0.50*
Group-VII	7.72 ± 0.21*	83.73 ± 3.43*	10.50 ± 1.06*	48.71 ± 2.70*	0.91 ± 0.32*

Values are given as mean ± SEM, $n=6$.

[a] $p < 0.05$ when diabetic control group were compared with normal control group.

* $p < 0.05$ when experimental groups were compared with diabetic control.

Table 5
Effect of HMELEO treatment for 45 days on superoxide dismutase, catalase, glutathione peroxidase, glutathione and lipid peroxidation in kidney of control and experimental groups of rats.

Groups	Kidney				
	SOD (units/mg protein)	CAT (μmol/min/mg protein)	GPx (μmol/min/mg protein)	GSH (mM/100 g tissue)	TBARS (μmol/100 g tissue)
Group-I	14.71 ± 0.31	72.29 ± 2.9	13.06 ± 1.02	36.71 ± 1.58	1.52 ± 0.21
Group-II	08.51 ± 0.22[a]	51.73 ± 3.0[a]	7.21 ± 0.99[a]	27.29 ± 1.61[a]	2.40 ± 0.27[a]
Group-III	13.15 ± 0.36*	71.14 ± 2.7*	11.47 ± 0.86*	33.41 ± 1.93*	1.63 ± 0.31*
Group-IV	09.99 ± 0.51	58.09 ± 2.8*	8.93 ± 1.10	30.90 ± 1.30	2.07 ± 0.52
Group-V	11.09 ± 0.67*	64.75 ± 3.67*	10.52 ± 1.54*	32.79 ± 1.43*	1.81 ± 0.63*
Group-VI	12.17 ± 0.72*	69.34 ± 3.26*	11.48 ± 1.10*	33.70 ± 1.38*	1.58 ± 0.79*
Group-VII	13.10 ± 0.50*	73.39 ± 3.53*	12.00 ± 1.31*	34.42 ± 1.17*	1.43 ± 0.92*

Values are given as mean ± SEM, $n=6$.

[a] $p < 0.05$ when diabetic control group were compared with normal control group.

* $p < 0.05$ when experimental groups were compared with diabetic control.

diabetic rats. The increased level of TBARS was found to be reverted back to near normal status after treatment of HMELEO (Tables 4 and 5). The HMELEO was found to possess antioxidant effect in a dose dependent manner.

4. Discussion

Medicinal plants are widely used by the population of developing countries as alternative therapy. In India, hundreds of plants are used traditionally for the management of diabetes mellitus. Unfortunately only a few of such Indian medicinal plants have received scientific scrutiny. The present study was therefore designed to study the antioxidant potential and hypoglycemic effect of *Emblica officinalis* Gaertn. leaves extract against streptozotocin induced diabetic rats. The continuous treatment of HMELEO for a period of 45 days produced a significant ($p < 0.05$) decrease in blood glucose level in diabetic rats which is comparable to that of standard and diabetic control group.

An increase in blood glucose seen in the oral glucose tolerance test (OGTT) was significantly greater in the diabetic rats than in the non-diabetic rats. The level of plasma insulin was increased by glucose tolerance test in the non-diabetic rats, while it was not changed in diabetic rats. Oral administration of HMELEO 400 mg/kg significantly improved the impaired glucose tolerance in the diabetic rats with change in plasma insulin level. Considering the above result, the hypoglycemic effect of the plant may involve its insulin-like action i.e., acting at peripheral level to increase cellular glucose uptake or increase glycogenesis. A number of plants have been shown to exert hypoglycemic activity through stimulation of insulin release (Prince and Menon, 2000) like glibenclamide that is reported to enhance the activity of beta cells of pancreas resulting in increased secretion of large amount of insulin which in turn brings down blood glucose level (Andrew, 2000). From the results it is assumed that the leaves extract of *Emblica officinalis* Gaertn. could be responsible for stimulation of insulin and the observed restoration of metabolic activity.

Diabetes is associated with weight loss. The reversal of weight loss in extract-treated diabetic group indicates that the restorative effect of HMELEO may be by the reversal of gluconeogenesis and glycogenolysis (Griesmacher et al., 1995). The decreased level of total hemoglobin in diabetic rats is mainly due to the increased formation of HbA1c. During diabetes mellitus, the excess glucose present in the blood reacts with hemoglobin to form HbA1c (Koening et al., 1976). The amount of HbA1c increase is directly proportional to the fasting blood glucose level (Al-yassin and Ibrahim, 1981). Administration of *Emblica officinalis* Gaertn. leaves extract to diabetic rats reduced the glycosylation of hemoglobin by virtue of its normoglycaemic activity and thus increase the levels of hemoglobin in diabetic rats. The concentrations of lipids, such as cholesterol, triglycerides (TG) and HDL-C, were significantly higher in diabetic rats than in the control group. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids (Rajalingam et al., 1993).

The elevation of biomarker enzymes such as SGOT, SGPT, and ALP was observed in diabetic control rats and indicates the hepatocellular damage (Jaeschkle et al., 2002). The present study also shows that injection of STZ induces hepatic damage that elevates intracellular enzymes, such as transaminases and alkaline phosphate. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activity (Ghosh and Suryawansi, 2001). The hepatic damage was restored hepatocytes and the elevated transaminases were significantly

reduced by HMELEO extract. From this point of view *Emblica officinalis* Gaertn. leaves extracts may act as hepatoprotective agent. The diabetic hyperglycemia induces elevation of the serum level of urea and creatinine which was considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). An increase in serum level of urea and creatinine levels in STZ-diabetic rats may indicate diminished ability of the kidney to filter these waste products from the blood and excrete them in the urine. On the other hand, the results indicates that treatment of diabetic group with *Emblica officinalis* Gaertn. leaves extract significantly reduced serum urea and creatinine level. Based on these findings, the extract of leaves of this plant may enhance the ability of the kidney to remove these waste products from the blood, as indicated by a protective effect on the kidney of diabetic rats.

Diabetes is strongly co-related with oxidative stress induction. Lipid peroxidation is one of the characteristic features of diabetes mellitus. Measurement of plasma thiobarbituric acid reactive substances (TBARS) was used as an index of lipid peroxidation and it helps to assess the extent of tissue damage (Gutteridge, 1995). Several studies have reported an increase in TBARS and hydroperoxides in plasma, liver and kidney in experimental diabetes mellitus (Venkateswaran and Pari, 2002; Ananthan et al., 2004). The result of the present study shows that *Emblica officinalis* Gaertn. leaves extract significantly ($p < 0.05$) decreases TBARS level and reduces the risk of tissue damage.

Oxidative stress in diabetes is coupled to a decrease in the antioxidant status, which can increase the deleterious effects of free radicals. The SOD and CAT are the two major scavenging enzymes that remove free radicals. Reduced activities of these antioxidant enzyme in liver, kidney and pancreas tissues have been observed in diabetic rats and this activity may result in a number of deleterious effects due to accumulation of superoxide anion (O) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (OH), resulting in initiation and propagation of LPO. SOD protects from oxygen free radicals by catalyzing the removal of superoxide radical, which damage the membrane and biological structures. Catalase was shown to be responsible for the detoxification of H_2O_2, and protects the tissues from highly reactive hydroxyl radicals (Mahboob et al., 2005). This decrease in CAT activity could result from inactivation by glycation of enzyme (Yan and Harding, 1997). In the present study, extract treated groups showed a significant increase in the hepatic and renal SOD and CAT activities of the diabetic rats. This means that the extracts can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes. This result clearly shows that *Emblica officinalis* Gaertn. leaves contain a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of superoxide radicals and hydrogen peroxide radical.

Glutathione is a tripeptide, intracellular antioxidant and protects the cellular system from adverse effects of lipid peroxidation. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (Winterbourn, 1995). Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased GSH content (Mohammed, 2008). Treatment with *Emblica officinalis* Gaertn. leaves extract resulted in the elevation of the GSH levels, which protects the cell membrane against oxidative damage by regulating the redox status of protein in the membrane (Inove et al., 1987).

Glutathione peroxidase (GPx), an enzyme with selenium, plays a primary role in minimizing oxidative damage. It works together with glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione (Bruce et al., 1982). Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme (Hodgson and Fridovich, 1975). Administration of *Emblica officinalis* Gaertn. leaves extract and glibenclamide increases the activities of GPx in the tissue of diabetic rats.

The recovery of antioxidant enzymes with HMELEO in treated group has been supported here by the diminution in the level of end products of lipid peroxidation as this is the good sensor for the assessment of oxidative stress. The fruits of *Emblica officinalis* Gaertn. show antioxidant and antidiabetic activity (Sabu and Kuttan, 2002) due to the presence of polyphenols like ellagic acid, gallic acid and ascorbic acid (Nampoothiri et al., 2011), tannins like emblicanin-A and emblicanin-B (Ghosal et al., 1996) and due to tannoids like kaempferol and quercetin (Bhattacharya et al., 1999). The leaves of the plant also contain ellagic acid, gallic acid, ascorbic acid, tannins emblicannin A and B (Thakur et al., 1989), and tannoids like kaempferol, kaempferol-3-o-glucoside and rutin (Duke, 1992). On the basis of this evidence it is possible that these activities of *Emblica officinalis* Gaertn. leaves are due to the presence of the above said phytoconstituents.

5. Conclusion

In conclusion, the present study showed that *Emblica officinalis* Gaertn. leaves extract possesses potent antioxidant activity, which may be responsible for its hypoglycemic property. From preliminary phytochemical analysis it was found that the major chemical constituents of the HMELEO were tannins, polyphenolic compound and flavonoids so it is possible that the presence of tannins or flavonoids may be responsible for the observed antidiabetic activity and reduce oxidative stress. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.

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