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In vivo Anti-Diabetic Activity of the Methanolic and Aqueous Bark Extracts of the Plant *Emblica officinalis* Gaertn

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Abstract: The present studies emphasize the investigation on methanolic and aqueous extract of *Emblica officinalis* Gaertn. (Family-Euphorbiaceae) stem barks for its anti-diabetic activity in animal model. Alloxan induced diabetes model was used for the study. The standardized doses of 250, 500, 1000 and 2500 mg kg^{-1} body weight of the extract were administered orally to normal and diabetic rats in order to define its hypo-glycemic potential. The effective dose (ED_{50}) was determined by the $1/10^{th}$ of maximum dose of methanolic and aqueous stem bark extract of *Emblica officinalis* respectively. Results depicted that the maximum fall of blood glucose level of normal rats were observed after 6 hour during fasting blood glucose studies, with the dose of 250 mg kg^{-1} identified as the most effective dose. However, the dose of 250 mg kg^{-1} of methanolic and aqueous stem barks extract in moderate and mild-diabetic animals produced a maximum fall blood glucose level of 45 and 44% ($p < 0.01$), respectively. The effect of bark extracts on serum lipid profile (cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein) were measured in diabetic rats. This evidence clearly indicates that the aqueous and methanolic extract of *E. officinalis* stem barks have significant hypoglycemic potential as well as anti-diabetic activity.

Key words: *Emblica officinalis* · Anti-diabetic · Wistar albino rats · Biochemical data

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism [1]. Insulin is a protein hormone secreted by the beta cells of islets of langerhans of pancreas. Deficiency of effective insulin in the body causes a disease called diabetes mellitus [2]. India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 [3]. Type I diabetes (Insulin dependent) is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from type-I are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to Insulin and can be treated with dietary changes, exercise and medication [4, 5].

The World Health Organization (WHO) has listed 21,000 plants, which are being used for medicinal purposes around the world. Among these 2500, species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [6].

The amla (vernacular name of *Emblica officinalis*) tree is native to tropical southeastern Asia, particularly in central and southern India, Pakistan, Bangladesh, Ceylon, Malaya, southern China and the Mascarene Islands. It is commonly cultivated in home gardens throughout India and grown commercially in Uttar Pradesh (UP). Many trees have been planted in southern Malaya, Singapore and throughout Malaysia. In India and to a lesser extent in Malaya, the amla is important and esteemed, raw as well as preserved and it is prominent in folk medicine. The tree is a graceful ornamental, normally reaching a height of 60 ft and in rare instances, 100 ft. It's fairly smooth bark is a pale grayish-brown and peels off in thin flakes like that of the guava. While actually deciduous, shedding its branchlets as well as its leaves, it is seldom entirely bare

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and is therefore often cited as an evergreen [7]. The fruits, fresh, dried or stewed act as a tonic, a diuretic and a laxative. The fruits are useful in treating diabetes, cough, asthma, bronchitis, intermittent fevers and cardiac disorders. The seeds are used to treat asthma and abdominal disorders. The powdered seeds are used to treat asthma, bronchitis and biliousness. The root is said to be an emetic. The root bark is also useful in treating ulcerative stomatitis. The bark of the plant also useful in treating gonorrhoea, jaundice, diarrhoea and myalgia. As a whole the extract of the fresh bark mixed with honey and turmeric, is being given to treat gonorrhoea. Syrup of the plant mixed with lemon juice is used to treat dysentery [8]. It is well known that the fruits contain lots of Vit-C and anti-oxidant. Generally, in the Indian traditional medicinal system, each parts of this plant have important role to cure the alignment. The present investigations were conducing with the stem bark extract (aqueous and methanolic) in relation to its' hypoglycaemic activity.

MATERIALS AND METHODS

Alloxan monohydrate was purchased from Sigma-Aldrich Co, Bangalore. Glucose, total cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Cholesterol and triglycerides (TG) were assayed using commercially available kits (SPAN Diagnostics). One-touch glucometer (Accu-chek sensor) and Uristix were purchased from Micro gene Diagnostic Systems Pvt. Ltd. New Delhi, India.

Collection and Authentication of Plant Material: The stem barks of *Emblica officinalis* Gaertn. (Family-Euphorbiaceous) were collected from campus garden of Dibrugarh university, Assam, India in the month of August 2011. The plant materials were identified and authenticated taxonomically by Dr. B.K. Sinha at Botanical Survey of India, Shillong. A voucher specimen DU/PSc/ HRB-01/2011 of the collected sample was preserved in the institutional herbarium and departmental museum for future references.

Preparation of Stem Barks Powder: The freshly collected stem barks of *Emblica officinalis* Gaertn. air-dried for 25 to 30 days at room temperature under the control conditions, reduced to coarsely powder through sieve no. 40 and stored in an airtight container.

Extraction of Stem Barks: The powdered stem barks were extracted with methanol and water by cold maceration process for 48 hours. The extract was then filtered and evaporated to dryness at 0-60°C under reduced pressure in a rotary evaporator. The dark brown mass was stored in a desicator for further uses.

Preliminary Phytochemical Screening: *E. officinalis* was subjected to preliminary phytochemical screening tests to verify the presence of alkaloids, terpenoids, reducing sugars, flavonoids, saponins glycosides and tannins according to the standard procedures [9, 10].

Experimental Animals: Wister albino rats (200-250g) were used for experimental purposes, maintained under 28-30°C temperature, 60-70% relative humidity and 12 hours day and night cycle. The animals were used with the approval of the Institute Animal Ethics Committee (Approval no: IAEC/DU/09, Regd. No. 1576/Go/a/11/CPCSEA dated 17.02.2012). Animals were fed a standard pellet (Lipton India, Ltd) and water. Animals described as fasted were deprived of food for 16 hours but had free access to water.

Acute Oral Toxicity Study: A total five female *Albino Wistar* rat were used, which received a single oral dose (2500 mg/kg body weight) of both extracts after overnight fasting. After administration of plant extracts, food was withheld for further 3-4 h. Animals were observed individually at least once during 30 min after dosing, periodically during the first 24h (with special attention during first 4h) and daily thereafter 14 days. At the end of the study the animals were observed for general toxic signs, morphological behaviour and mortality. One tenth of LD_{50} (2500 mg/kg body weight) was taken for further study [11].

Experimental Design of Anti-diabetic Activity: The vehicles and the drugs were administered to animals orally using an intragastric tube daily for three weeks. Overnight fasted normal animals were randomly divided into five groups of five rats in each group. The group I served as control, which received vehicle i.e. 0.5% CMC solution. Group II diabetic control which received alloxan monohydrate (100 mg/kg body weight), group III and IV were treated orally with methanolic stem bark extract (MSBE) and aqueous stem bark extract (ASBE) at 250 mg/kg body weight respectively. Group V received metformin hydrochloride 5 mg/kg body weight orally as standard drug. Fasting blood glucose (FBG) was determined by the blood Glucose meter and strips [12].

Induction of Diabetes and Blood Glucose Estimation: Overnight fasted albino rats were made diabetic by injecting alloxan monohydrate intraperitonially at a dose of 100 mg/kg body weight. Alloxan was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was confirmed in alloxan injected rats by measuring the fasting blood glucose concentration, 72 hr after the alloxanization. Rats with blood glucose level above 250 mg/dl were considered diabetic and were used in this study.

Blood samples were then collected for determination of blood glucose level on 0, 7, 14, 21 days. The rats were fasted overnight and blood was withdrawn by rupturing the tail vein. The blood glucose levels were measured by one touch glucometer and Blood glucose check up strips throughout the three weeks of treatment.

Biochemical Estimation: At the end of 21st day, after the estimation of blood glucose level, the animals were sacrificed by decapitation. Blood was collected by cardiac puncture and serum was separated for determination of biochemical parameters, the level of serum triglyceride (TG), high-density lipid cholesterol (HDL), low-density lipid cholesterol (LDL) and total cholesterol (TC) were estimated by using commercial available kits. (SPAN Diagnostics kit).

RESULTS

Acute Toxicity Test: No mortality was recorded among the rats at the dose of 2500 mg/kg. Hence, one tenth of the dose tested, i.e., 250 mg/kg body weight was selected for the study.

Change in Body Weight: The changes of body weight of the alloxanized rats were decreased gradually; on the other hand, the animals of group IV (ASEB) improved their body weight progressively during the long 21 days of treatment. The animals from group III (MSBE) became healthier throughout the treatment except in the 7th day, but the group V animals showed the improvement of body weight gradually as in normal control group (group I).

Change in Blood Glucose Level: The effects of methanolic and aqueous stem bark extracts of *Emblica officinalis* were investigated in the alloxan induced diabetic rats using metformin hydrochloride as standard. The mean blood glucose concentration of controlled and

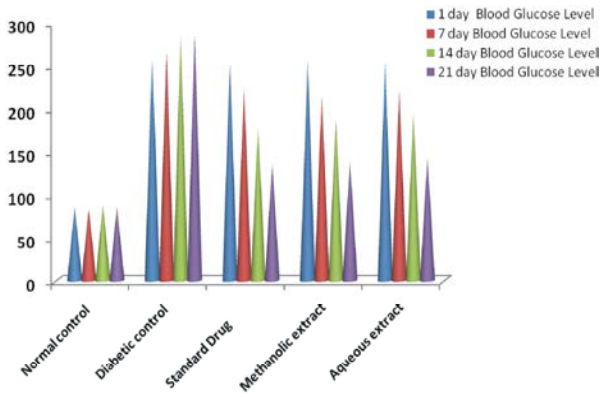

Fig. 1: Chart shows the blood glucose level on the day of 1st, 7th, 14th and 21st day

drug treated groups were estimated on 0, 7, 14 and 21st day of the treatment. Hypoglycemia observed when the animals were treated with MSBE, ASBE and standard drugs (Fig. 1).

Change in Biochemical Parameters: Serum cholesterol, serum tryglycerides, serum HDL levels were decreased by metformin hydrochloride and all the extracts of *E. officinalis* due to 21 days of treatment. The LDL levels were significantly failed down to 22.6 mg/dl of ASBE, in comparison to standard drug but the MSBE groups data remains parallels.

DISCUSSION

Diabetes is one of the leading diseases around the globe. Management of diabetes is being a tough task with the synthetic medicines as they have many side effects. The interest has been increased on the medicinal plants used for remedy or reducing the risk of diseases. In the recent scenario, the scientists have emphasized for the herbal extracts and initiated extensive research to observe their effective and protective role in the diseased animal models. The present results indicate significant increase in body weight and decrease in blood glucose levels in diabetic rats during 21 days of study and they became normal when treated with the plant extracts i.e. MSBE & ASBE. Significant increased of body weight were noted from day 1st to 21st days i.e. 233.16 gm to 246.50 gm and 232 gm to 242.16 gm in case of MSBE and ASBE respectively. On the other side the gradual fall of blood glucose value were observed from 255.34 to 138.00 and 253.60 to 141.67 respectively in the duration of 1st day to 21st days. This suggests that the plant *Emblica officinals* have protective

Table 1: Grouping of experimental animals

GROUP-I	Normal control and received vehicle i.e. 0.5% CMC solution orally.
GROUP-II	Diabetic control and received 100 mg/kg body weight alloxan monohydrate.
GROUP-III	Treated orally with MSBE, 250mg/kg body weight.
GROUP-IV	Treated orally with ASBE, 250mg/kg body weight.
GROUP-V	Metformin hydrochloride 5mg/kg body weight, orally on 3rd day after alloxanation.

Table 2: The effect of MSBE and ASBE of *Embilica officinalis* on body weight (gm) in alloxan- induced diabetic rats

Treatment	1st day	7 days	14 days	21 days
Normal control (GROUP-I)	230.83±0.91$^{\#\#}$	238.33±0.39	247.16±0.69$^{\#\#}$	253.66±0.49$^{\#\#}$
Diabetic control (100 mg/kg Alloxan) (GROUP- II)	222.50±0.54$^{**\#\#}$	193.16±1.19$^{**\#\#}$	183.66±0.89$^{**\#\#}$	174.50±1.09$^{**\#\#}$
MSBE (250 mg/kg) (GROUP-III)	233.16±0.42$^{\#\#}$	230.16±0.85**	237.33±0.86$^{**\#\#}$	246.50±1.53$^{**\#\#}$
ASBE (250 mg/kg) (GROUP-IV)	232.00±0.60$^{\#\#}$	234.50±0.41$^{**\#\#}$	239.00±0.48$^{**\#\#}$	242.16±1.34**
Standard Drug (Metformin hydrochloride 5mg/kg) (GR0UP-V)	227.50±0.73**	228.66±0.63**	232.83±0.55**	238.66±0.82**

Values are mean± SEM (n=6), statistical significance: *P<0.05, **P<0.01, compared with vehicle group I; $^{\#}$P<0.05 $^{\#\#}$P<0.01, compared with standard drug group V.

Table 3: The effect of MSBE and ASBE of *Embilica officinalis* on fasting blood glucose level in alloxan- induced diabetic rats

Treatment	1stday	7thday	14thday	21st day
Normal control (GR0UP-I)	85 ± 0.33$^{\#\#}$	82.33± 0.83$^{\#\#}$	87.25 ±0.60$^{\#\#}$	85.33±1.17$^{\#\#}$
Diabetic control (100 mg/kg Alloxan) (GR0UP-II)	256 ± 0.50$^{**\#\#}$	265 ± 0.33$^{**\#\#}$	283.33±0.34$^{**\#\#}$	285 ± 1.33$^{**\#\#}$
MSBE (250 mg/kg) (GROUP-III)	255.34 ±1.34$^{**\#\#}$	214.34±1.95$^{**\#\#}$	185.67±1.34$^{**\#\#}$	138 ± 0.99**
ASBE (250 mg/kg) (GROUP-IV)	253.6± 1.01**	220.66±1.50**	193.34±3.39$^{**\#\#}$	141.67±1.24$^{**\#\#}$
Standard Drug (Metformin hydrochloride5mg/kg) (GR0UP-V)	251.34± 0.50**	222.34±0.69**	175±0.33**	134.34±1.01**

Values are mean± SEM (n=6), statistical significance: *P<0.05, **P<0.01, compared with vehicle group I; $^{\#}$P<0.05 $^{\#\#}$P<0.01, compared with standard drug group II.

Table 4: Effect of MSBE and ASBE of *Emblica officinalis* on cholesterol, triglycerides, HDL and LDL of control and experimental rats

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Normal control (GR0UP - I)	116.34 ± 0.19$^{\#\#}$	83 ± 1.76$^{\#\#}$	42 ± 0.99$^{\#}$	23.2 ± 0.73$^{\#\#}$
Diabetic control (100 mg/kg Alloxan monohydrate) (GR0UP-II)	244 ± 0.33$^{**\#\#}$	100.67± 1.49$^{**\#\#}$	35.34± 1.34**	31.6 ± 1.06**
MSBE (250 mg/kg) (GROUP-III)	139 ± 1.33$^{**\#\#}$	104.67± 1.17$^{**\#\#}$	53.34 ±1.34$^{**\#}$	33.4 ± 0.89
ASBE (250 mg/kg) (GROUP-IV)	142.34± 0.19**	106.34± 0.69$^{**\#\#}$	49 ± 1.33**	22.6 ± 0.99$^{\#\#}$
Standard Drug (Metformin hydrochloride5mg/kg) (GR0UP-V)	144.34± 0.19**	113 ± 1.33$^{**\#\#}$	47 ± 1.66*	32.5 ± 1.31**

Values are mean± SEM (n=6), statistical significance: *P<0.05, **P<0.01, compared with vehicle group I; $^{\#}$P<0.05 $^{\#\#}$P<0.01, compared with standard drug group II

role in reducing glucose levels as well as in increasing body weight. This study confirms the traditional belief that *Emblica officinals* bark has anti diabetic effect. In the metformin treated diabetic rats the value fall from 251to 222 mg/dl on 7th day and drastically decreases to 175 on 14th and 134 on 21st day. This indicates that metformin hydrochloride is more potent when compared with both MSBE & ASBE and MSBE is more potent when compared to ASBE.

The effect of MSBE and ASBE of *Emblica officinalis* on body weight and on fasting blood glucose level in alloxan-induced diabetic rats along with standard deviation (SD) values are given in Table 2 and 3. Administration of Alloxan (100 mg/kg, i.p.) led to three-fold elevation of fasting blood glucose levels, which was maintained over a period of three weeks. Three weeks of daily treatment of MSBE and ASBE of *Emblica officinals* led to a dose-dependent fall in blood sugar levels by 45% and 44% respectively. Effect seems to reach maximum after 21 days of treatment. Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 21 days (Table 2).

The effect of stem bark extract on cholesterol, triglyceride, HDL and LDL level in alloxan induced diabetic rats were given above in Table 4. The lower dose of methanolic and aqueous stem bark extract 250mg/kg produced a significant decrease (in fasting blood glucose level on 21st day) in cholesterol level whereas slight increase is seen in triglyceride and HDL level when compared with diabetic controlled animal. These observations provide supportive evidence on the anti-diabetic potency of the methanolic and aqueous stem bark extract of *Emblica officinalis* Gaertn.

Statistical Analysis: All result were expressed as the mean± standard error of mean (SEM). The results were analyzed for statistical significance by One-way Analysis of Variance (ANOVA) followed by Dunnett's tests with the help of Graph-Pad Prism software. The significance was expressed by *P* value, as mention in the Tables. P<0.01 was considered as statistically significant [13].

CONCLUSIONS

The methanolic and aqueous extracts of *Embilica officinalis* Gaertn. Bark exhibited significant anti-hyperglycemic activities in alloxan-induced diabetic rats. These extracts showed improvement in parameters like body weight and lipid profile and so might be of value in diabetes treatment.

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