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RESEARCH ARTICLE

# Anti-diabetic activity of *Emblica officinalis* in animal models

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## Abstract

The aqueous extract of *Emblica officinalis* Gaertn. (syn: *Phyllanthus emblica* L.) (Euphorbiaceae) seeds was investigated for its anti-diabetic activity in animal models. Streptozotocin (STZ)-induced type 2 diabetes models were used for the study. The standardized doses of 100, 200, 300, and 400 mg kg<sup>-1</sup> body weight of the extract were administered orally to normal and diabetic rats in order to define its glycemic potential. The maximum fall of 27.3% ( $p < 0.001$ ) in the blood glucose level of normal rats was observed at 6 h during fasting blood glucose studies, with the dose of 300 mg kg<sup>-1</sup> identified as the most effective dose. The same dose produced a fall of 25.3% ( $p < 0.001$ ) in the same models during the glucose tolerance test (GTT) at 3 h after glucose administration. However, the dose of 300 mg kg<sup>-1</sup> of aqueous seed extract in sub- and mild-diabetic animals produced a maximum fall of 34.1 and 41.6% ( $p < 0.01$ ), respectively, during the GTT at 3 h after glucose administration. This evidence clearly indicates that the aqueous extract of *E. officinalis* seeds has definite hypoglycemic potential as well as anti-diabetic activity.

**Keywords:** *Emblica officinalis*; anti-diabetic; albino Wistar rats; glucose tolerance test

## Introduction

The disease diabetes is well known in each and every part of the world. Type 2 diabetes mellitus (T2DM) is a major and growing health problem throughout the world. T2DM results from both peripheral insulin resistance and impaired insulin secretion. Insulin resistance arises as a consequence of obesity, a sedentary life, and aging, resulting in hyperglycemia and diabetes. Blood pressure elevation and dyslipidemia are collectively called “metabolic syndrome X” (Arulmozhi & Portha, 2006). As diabetes is a global burden, its management should be explored systematically and scientifically. This is the main reason for the persistent interest all over the world to explore remedies from the so-called “alternative system of medicine.” Since the natural remedies are of low toxicity with minimal or no side effects (Rai et al., 2007; Singh et al., 2007), therefore medicinal plants are of significant interest to scientists. It is important to justify the various uses of medicinal

plants enunciated in traditional systems of medicine, scientifically.

*Emblica officinalis* Gaertn. (syn: *Phyllanthus emblica* L.) (Euphorbiaceae) grows in tropical and subtropical parts of China, India, and the Malay Peninsula. The fruit is commonly known as amla or emblic myronalan, and is of high value in traditional Indian medicine. Several constituents of *E. officinalis* have been identified: the hydrolyzable tannins and its derivatives are the key ingredients (Chaudhuri, 2004). The two derivatives emblicanin A and B have been proposed to be the active constituents, with significant *in vitro* antioxidant activity (Ghosal et al., 1996). The fruits of *E. officinalis* have been reported to have potent antimicrobial (Ahmad et al., 1998), antioxidant (Bhattacharya et al., 1999; Babu et al., 2004; Rao et al., 2005), adaptogenic (Rage et al., 1999), hepatoprotective (Jeena et al., 1999), anti-tumor (Jose et al., 2001), and antiulcerogenic (Sairam et al., 2002; Kumar et al., 2004) activities. Its antioxidant activity is related to the presence of gallic and

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ellagic acids that inhibit the degradation of vitamin C (Damodaran & Nair, 1936). Since the seeds of *E. officinalis* have not been explored scientifically for their role in diabetes management, these were our choice for the present study. This report on the anti-diabetic effect of the aqueous extract of *E. officinalis* seeds is the first from our research group using experimental models.

## Materials and methods

### *Plant material and extraction procedure*

The fruits of *E. officinalis* (15 kg) were purchased during the month of November from the local market of Allahabad, India and authenticated by Professor Satya Narayan, Taxonomist Department of Botany, University of Allahabad, India. A voucher specimen has been submitted to the University herbarium. The fruits were boiled at 70°C and pulps of fresh fruits were separated to take out the hard seeds. The shade-dried seeds (2.5 kg) were mechanically crushed and extracted with distilled water (65°C) using Soxhlet apparatus for up to 72 h. The extract was filtered and concentrated in a rotary evaporator at 45–50°C under reduced pressure to obtain semi-solid material, which was then lyophilized to obtain a powder (yield 12.3%, w/w) (Rai et al., 2007, 2008).

### *Chemicals*

Streptozotocin was purchased from Sigma-Aldrich Co., USA. Blood glucose level (BGL) for fasting blood glucose (FBG) and glucose tolerance test (GTT) studies was assayed using kits from Bayer Diagnostics, India and a one touch Accu-Chek sensor from Roche Diagnostics, Germany. The solvents were from E. Merck.

### *Preliminary phytochemical screening*

Preliminary phytochemical screening was carried out for phenols and flavonoids (Kokate, 1994; Harborne, 1998). Phenolic extraction of the dried powder sample was performed using 70% ethanol, and the total phenolic content was analyzed using Folin-Ciocalteu reagent (McDonald et al., 2001).

### *Experimental animals*

More than 100 male albino Wistar rats of the same age group with body weight 150–200 g were selected for all experiments. The animals, obtained from the National Institute of Communicable Disease (NICD), New Delhi, India, were housed in polypropylene cages at an ambient temperature of 25–30°C and 45–55% relative humidity, with a 12 h each dark/light cycle. Animals were fed

a pellet diet (Pashu Ahar Kendra, Varanasi) and water *ad libitum*. The study was approved by the Institutional Ethics Committee.

### *Induction of diabetes*

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) 50 mg kg<sup>-1</sup> (Sigma-Aldrich, Seelze, Germany) in 0.1 M citrate buffer (pH = 4.5) (El-Fiky et al., 1996) to rats deprived of food overnight. After 3 days of STZ administration, rats with marked hyperglycemia were selected for the study (Rai et al., 2008). The rats with stable hyperglycemia were divided into two groups of 36 rats each:

- sub-diabetic animals with normal FBG (80–120 mg dL<sup>-1</sup>) and abnormal postprandial glucose (PPG; >210 mg dL<sup>-1</sup>) levels, also known as streptozotocin recovered (SR);
- mild-diabetic animals with FBG 150–200 mg dL<sup>-1</sup> and PPG >250 mg dL<sup>-1</sup>.

### *Blood glucose estimation*

BGL was estimated by the glucose oxidase method (Barham & Trinder, 1972) using a standard kit from Bayer Diagnostics India Ltd.

### *Experimental design*

Initial screening of the aqueous extract for hypoglycemic activity was done with a range of variable doses in normal healthy rats by conducting FBG and GTT studies. The anti-diabetic effect was assessed in sub- as well as mild-diabetic models (Shukla et al., 1994; Rai et al., 2008) with the same range of doses based on similar studies of FBG and GTT (Rai et al., 2008).

### *Assessment of hypoglycemic activity by FBG studies in normal healthy rats*

Five groups of six rats each, fasted overnight, were used in the experiment. Their FBGs were taken. Group I served as untreated controls and received vehicle (distilled water) only, and animals of groups II, III, IV, and V received aqueous seed extract suspended in distilled water at doses of 100, 200, 300, and 400 mg kg<sup>-1</sup>, respectively. Blood samples were collected from the tail vein at 2, 4, 6, and 8 h after treatment.

### *Assessment of hypoglycemic activity by GTT studies in normal healthy rats*

Varied doses of aqueous extract and vehicle treatment were given orally to different groups of normal healthy rats in the same fashion as above, after taking their FBG. The BGL value at 2 h after treatment was considered as

the "0" h value. The animals were then orally administered with  $2 \text{ g kg}^{-1}$  of glucose, and their glucose tolerance was studied at 1 h intervals for another 3 h. Thus, the total period of blood collection was up to 5 h.

#### Assessment of anti-diabetic activity by GTT in sub-diabetic rats

The rats were divided into six groups of six rats each. Group I was the control group, and received vehicle (distilled water) only, whereas varied doses of 100, 200, 300, and  $400 \text{ mg kg}^{-1}$  of extract were given orally to groups II, III, IV, and V, respectively, after taking their FBG. Blood glucose levels were then checked after 2 h of treatment, considered as the 0 h value, and then  $2 \text{ g kg}^{-1}$  glucose was given orally to all the groups. Blood glucose levels were further checked up to 3 h at regular intervals of 1 h each, considered as 1, 2, and 3 h values. The results were compared with those of group VI that served as the positive control group, treated with  $250 \text{ mg kg}^{-1}$  of tolbutamide, a reference drug.

#### Assessment of anti-diabetic activity by GTT in mild-diabetic rats

Varied doses of aqueous extract and vehicle treatment were given orally to different groups of mild-diabetic rats in the same fashion as above after taking their FBG. Blood glucose levels were then checked after 2 h of treatment, considered as the 0 h value, and then  $2 \text{ g kg}^{-1}$  glucose was given orally to all the groups. Blood glucose levels were further checked up to 3 h at regular intervals of 1 h each, considered as 1, 2, and 3 h values. The results were compared with those of group VI of rats, which were treated with  $250 \text{ mg kg}^{-1}$  of tolbutamide (hypoglycemic agent).

#### LD<sub>50</sub> experiment

The toxic effect of the water extract was also studied by LD<sub>50</sub> experiment. Two groups of rats of both sexes (six animals per group, three females and three males), weighing about 180–200 g, were orally administered a single dose of 3 and 4.5 g, respectively, of the aqueous extract. Then the rats were observed for gross neurologic (Cartmell et al., 1991) and toxic effects continuously.

Food consumption, feces, and urine were also examined at 2 h and then at 6 h intervals for 24 h.

#### Statistical analysis

Data were statistically evaluated using two-way analysis of variance (ANOVA), followed by *post hoc* Scheffe's test using the statistical package PRISM version 3.0. The values were considered significant when  $p < 0.05$ .

## Results

#### Effect on normal healthy rats

Table 1 describes the hypoglycemic effect of a single oral administration of varied doses of 100, 200, 300, and  $400 \text{ mg kg}^{-1}$  of aqueous seed extract in normal healthy rats. Treated rats showed a regular increase in percentage fall of 18.3 and 27.3% ( $p < 0.001$ ) with the doses of 100 and  $300 \text{ mg kg}^{-1}$ , respectively, after 6 h. However, a fall of only 22.8% ( $p < 0.001$ ) was observed with the dose of  $400 \text{ mg kg}^{-1}$  after the same interval of time, indicating thereby a slight decrease in the maximum fall observed with the dose of  $300 \text{ mg kg}^{-1}$ .

#### Effect on normal rats during GTT

Table 2 deals with the study of the aqueous extract of *E. officinalis* seeds on BGL levels and glucose tolerance of normal healthy rats. Different doses of 100, 200, 300, and  $400 \text{ mg kg}^{-1}$  of extract were given orally to overnight fasted healthy rats, after taking their FBG sample. The dose of  $300 \text{ mg kg}^{-1}$  produced a maximum fall of 25.3% ( $p < 0.001$ ) in BGL of rats after 3 h of glucose administration, whereas falls of 20.9, 22.9, and 22.1% ( $p < 0.001$ ) were observed in BGL with the doses of 100, 200, and  $400 \text{ mg kg}^{-1}$ , respectively.

#### Effect on sub-diabetic rats during GTT

Figure 1 demonstrates the anti-diabetic effect of varied doses of aqueous extract of *E. officinalis* seeds on

**Table 1.** Effect of varied doses of *E. officinalis* seed aqueous extract on blood glucose level (BGL) during fasting blood glucose (FBG) test of normoglycemic rats (mean  $\pm$  SD).

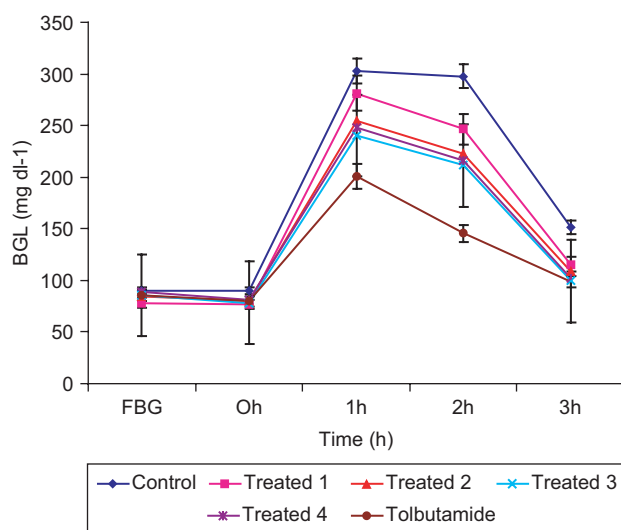
Experimental group	Treatment ( $\text{mg kg}^{-1}$ b.w.)	Pretreatment FBG	Blood glucose level ( $\text{mg/dL}$ )			
			Post-treatment (h)			
			0	2	4	6
Control	Distilled water	$74.2 \pm 3.2$	$74.1 \pm 3.8$	$73.2 \pm 4.2$	$74.7 \pm 3.3$	$74.1 \pm 3.7$
Treated 1	100	$73.2 \pm 3.6$	$71.7 \pm 5.7$	$63.2 \pm 4.6$	$60.7 \pm 3.5$	$59.8 \pm 4.2^*$
Treated 2	200	$72.9 \pm 3.9$	$70.6 \pm 4.9$	$66.3 \pm 3.5$	$60.1 \pm 3.8^*$	$59.8 \pm 5.1^*$
Treated 3	300	$73.5 \pm 4.2$	$70.1 \pm 5.1$	$62.7 \pm 3.7$	$54.6 \pm 4.4^*$	$53.4 \pm 4.6^*$
Treated 4	400	$75.8 \pm 4.5$	$72.5 \pm 3.2$	$66.1 \pm 4.2$	$59.7 \pm 5.1$	$58.5 \pm 4.3$

\* $p < 0.01$  as compared with initial value.

**Table 2.** Effect of varied doses of *E. officinalis* seed aqueous extract on blood glucose level (BGL) during glucose tolerance test (GTT) of normoglycemic rats (mean  $\pm$  SD).

Experimental group	Treatment (mg kg <sup>-1</sup> b.w.)	Blood glucose level (mg/dL)				
		Pretreatment FBG	Post-treatment (h)			
			0	1	2	3
Control	Distilled water	73.2 $\pm$ 4.5	73.7 $\pm$ 4.9	108.9 $\pm$ 3.7	103.1 $\pm$ 4.5	95.3 $\pm$ 4.1
Treated 1	100	74.7 $\pm$ 3.7	72.8 $\pm$ 4.1	89.4 $\pm$ 3.9	82.5 $\pm$ 4.4	75.3 $\pm$ 3.2*
Treated 2	200	75.1 $\pm$ 3.4	72.5 $\pm$ 4.4	88.6 $\pm$ 5.2	79.9 $\pm$ 4.6*	73.4 $\pm$ 3.9*
Treated 3	300	76.4 $\pm$ 4.2	73.2 $\pm$ 3.6	85.1 $\pm$ 3.4	78.3 $\pm$ 3.5**	71.2 $\pm$ 4.6*
Treated 4	400	72.7 $\pm$ 4.6	69.6 $\pm$ 3.9	86.2 $\pm$ 3.1	83.1 $\pm$ 4.7**	74.2 $\pm$ 4.9

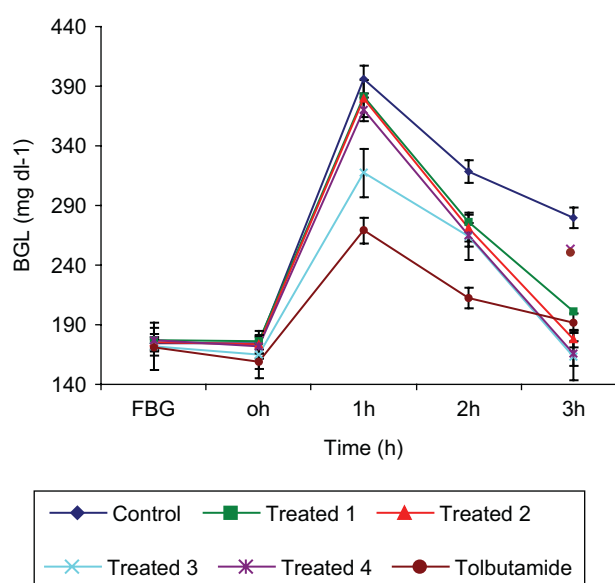
\* $p < 0.01$ , \*\* $p < 0.001$  as compared with control.

**Figure 1.** Effect of varied doses of *E. officinalis* seed aqueous extract on BGL during GTT in sub-diabetic rats. \* $p < 0.01$  as compared with control. Control: distilled water; Treated 1: 100 mg kg<sup>-1</sup>; Treated 2: 200 mg kg<sup>-1</sup>; Treated 3: 300 mg kg<sup>-1</sup>; Treated 4: 400 mg kg<sup>-1</sup>; Tolbutamide: 250 mg kg<sup>-1</sup>.

sub-diabetic rats. The standard drug tolbutamide 250 mg kg<sup>-1</sup> was also given as a reference drug. The fall observed was 23.6, 28.5, 34.1, and 32.4% ( $p < 0.001$ ) after 3 h of glucose administration with doses of 100, 200, 300, and 400 mg kg<sup>-1</sup>, respectively. However, the dose of tolbutamide showed the highest fall of 51.2% ( $p < 0.001$ ) at 2 h. Moreover, the fall observed at 3 h with this reference drug was 34.7% ( $p < 0.01$ ), which is comparable to the fall shown by the 300 mg kg<sup>-1</sup> dose of extract. Hence, this dose of extract can be identified as the most effective dose.

#### Effect on mild-diabetic rats during GTT

Figure 2 depicts the anti-diabetic potential of graded doses of aqueous extract of *E. officinalis* seeds and tolbutamide at a dose of 250 mg kg<sup>-1</sup> in mild-diabetic animals. The maximum fall observed after 3 h of glucose administration was 27.9, 36.3, 41.6, and 40.7% ( $p < 0.01$ ) with the doses of 100, 200, 300, and 400 mg kg<sup>-1</sup>,

**Figure 2.** Effect of varied doses of *E. officinalis* seed aqueous extract on BGL during GTT in mild-diabetic rats. \* $p < 0.001$  as compared with control. Control: distilled water; Treated 1: 100 mg kg<sup>-1</sup>; Treated 2: 200 mg kg<sup>-1</sup>; Treated 3: 300 mg kg<sup>-1</sup>; Treated 4: 400 mg kg<sup>-1</sup>; Tolbutamide: 250 mg kg<sup>-1</sup>.

respectively. Tolbutamide produced a maximum fall of 33.2% ( $p < 0.001$ ) within 2 h, which came down further to 31.3% ( $p < 0.01$ ) after 3 h. Hence, in mild-diabetic cases, the fall shown by the 300 mg kg<sup>-1</sup> dose of extract was much higher than that of the reference drug at 3 h.

#### LD<sub>50</sub>

The experiment was carried out on normal healthy rats. The behavior of the treated rats appeared normal. No toxic effect was reported at doses up to 10 and 15 times the effective dose of aqueous extract and there was no death in any of these groups.

#### Phytochemical studies

The preliminary studies indicated the presence of flavonoids and gallic acid. The total phenolic content in



terms of gallic acid equivalent ( $\text{mg g}^{-1}$  of dry mass) was  $21 \text{ mg g}^{-1}$  in the extract powder.

## Discussion

*E. officinalis* fruits are well known for their pharmacological activities (Ahmad et al., 1998; Bhattacharya et al., 1999; Jeena et al., 1999; Jose et al., 2001; Sairam et al., 2002) as well as antidiabetic properties (Salu & Kuttan, 2002; Suryanarayana et al., 2007). However, there are no reports on hypoglycemic and anti-diabetic effects of the seeds of *E. officinalis*, though it is well documented that the seeds have much more anti-diabetic activity than the fruits (Kamalakkannan & Prince, 2003; Kesari et al., 2006; Rai et al., 2008).

Thus, the present study based on FBG and GTT was carried out with graded doses of 100, 200, 300 and  $400 \text{ mg kg}^{-1}$  of aqueous extract of *E. officinalis* seeds given to normal as well as sub- and mild-diabetic rats. Results indicate that each dose of *E. officinalis* aqueous extract reduces the blood glucose level and improves glucose tolerance in both normal and diabetic animals.

It is evident from Tables 1 and 2 that the dose of  $300 \text{ mg kg}^{-1}$  is the most effective dose as it showed the maximum reduction in FBG within 6 h and maximum improvement in glucose tolerance after 3 h of glucose administration, respectively, in normal rats. Since the animals were fasted overnight before treatment, therefore the hypoglycemic action of the extract by inhibiting glucose absorption (Adamson & Okafor, 1990; Anderson & Akanji, 1991) can be ruled out. Moreover, in the case of sub- and mild-diabetic animals the maximum glucose tolerance was also associated with the same dose of  $300 \text{ mg kg}^{-1}$  at 3 h (Figures 1 and 2). These results are comparable with those of the standard drug tolbutamide, which showed practically the same percentage of fall in BGL at the same interval of time.

Since it is well documented that the administration of STZ selectively destroys the  $\beta$  cells of the pancreas and causes hyperglycemia in rats (Goldner & Gomori, 1943; Hofteizer, 1973; Rai et al., 2008), therefore the antihyperglycemic effect of the aqueous extract of the seeds might be due to the activation of existing  $\beta$  cells of the pancreas (Kar et al., 1999; Rai et al., 2008). This plausible action of the extract may be confirmed by the well established mechanism of action of the standard drug tolbutamide, i.e. activation of  $\beta$  cells (Henquin, 1980), in STZ-induced diabetic rats.

Preliminary phytochemical screening revealed the presence of flavonoids and gallic acids in the extract. The phenolic content in terms of gallic acid equivalent

is  $21 \text{ mg g}^{-1}$ . Moreover, flavonoids (Anila & Vijayalakshmi, 2000, 2002) and tannoids (Suryanarayana et al., 2007) isolated from the fruits of *E. officinalis* are also reported to manage diabetes and its complications.

Further exploration of the most effective dose ( $300 \text{ mg kg}^{-1}$ ) in STZ-induced severely diabetic models (type 1) along with the isolation of bioactive principles from active fractions is in progress.

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