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Anti-hyperglycaemic effect of *Emblica officinalis* fruits to Swiss Albino Mice

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Abstract
Emblica officinalis locally known as 'Amloki' has medicinal properties and used in the formulation of different Unani and Ayurvedic Medicine. Major bioactive constituents of *E. officinalis* are gallic acid, ascorbic acid, ellagic acid, rutin, quercetin, and catechol. It has a good reputation in Bangladesh, India and many other countries of the world as folk medicine for the treatment of a variety of diseases.
Diabetes mellitus is a metabolic disease characterized by hyperglycemia. Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and eventually the failure of organs. Synthetic antidiabetic agents can produce serious side effects. In view of the adverse effects associated with the synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycaemic agents from medicinal plants have become more important. Therefore, the present study was designed to investigate anti-hyperglycaemic effect of those plants with different doses. The anti-hyperglycaemic properties of methanolic extract of the *Emblica officinalis* fruits was evaluated in Swiss albino mice in order to scientifically validate its traditional therapeutic use with the different doses as per body weight.
In this experiment, among all the doses, the methanolic extract of *Emblica officinalis* fruit decrease the blood sugar level. The low dose (50 mg/kg body weight) decrease the glucose level but not as much as standard value. Oral administration of methanolic extract of *Emblica officinalis* fruit doses equivalent to 100, 200 and 400 mg/kg body weight produce significant anti-hyperglycaemic effects respectively.

Keywords: Anti-hyperglycaemic, methanolic extract, *Emblica officinalis*

Introduction
Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is well documented that chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and eventually the failure of organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Synthetic antidiabetic agents can produce serious side effects and they are not suitable for use during pregnancy [2]. In view of the adverse effects associated with the synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored [3]. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycaemic agents from medicinal plants have become more important (WHO, 1980) [4].
Emblica officinalis, is a member of the small genus of Emblica (Euphorbiaceae), Commonly known as Amloki in Bangladesh and Indian gooseberry or amla in India. It is an important dietary source of vitamin C, minerals and amino acids, and also contains phenolic compounds. The aqueous fruit extract of *Emblica officinalis* has been reported to exhibit hypolipidemic, antidiabetic and anti-inflammatory activities [5]. In the past decade, the aqueous fruit extract of *Emblica officinalis* has been tested for various pharmacological activities. Some of these activities include antioxidant properties, and it has also been reported that the aqueous fruit extract of *Emblica officinalis* is a potent inhibitor of lipid peroxide formation and a scavenger of hydroxyl and superoxide radicals *in vitro* [6, 7].
The present study includes a comparative assessment of the anti-hyperglycaemic potential of the methanolic extract of *Emblica officinalis* fruits and a commonly used antidiabetic drug to hyperglycaemic Swiss Albino Mice.

From the glucose levels analysis by glucometer after 120 minutes glucose gavaging, we found that the control mean was 6.7 mmol/l and the glibenclamide treated sample mean was 3.42 mmol/l. On the other hand, the *Emblica officinalis* fruits MeOH extract low dose (50 mg/kg) treated mean was 5.56 mmol/l, moderate dose (100 mg/kg) treated mean was 5.08 mmol/l, medium dose (200 mg/kg) treated mean was 4.40 mmol/l and high dose (400 mg/kg) treated mean was 3.78 mmol/l. Comparing with control mean all doses significantly decrease the blood sugar level.

Methods and Materials

Plant material collection

Fruits of *Emblica officinalis* were collected from Hamdard University Bangladesh campus in Gazaria, Munshiganj. The fruits of *Emblica officinalis* were identified by a Unani Medicine expert of Hamdard University Bangladesh.

Preparation of methanolic extract of fruits of *Emblica officinalis*

For preparation of methanol extract of fruits of *Emblica officinalis*, fruits were thoroughly sliced, dried in the shade, and pulverized into a fine powder. 100 g of the powder was extracted with 500 ml methanol over 48 hours. Methanol was evaporated at 40 °C and the extract was dissolved in Tween 20 prior to administration to mice by gavaging. The final weight of extract was 5.28 g.

Chemical and Drugs

We acquired glucose and glibenclamide from Square Pharmaceuticals Ltd. in Bangladesh. The other substances were all analytically graded. Strips and the glucosemeter were bought from BMA Bhaban, Dhaka, Bangladesh.

Animals

In the current investigation, Swiss albino mice weighing 12-15 g were employed. The International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B) provided the animals. Before the real studies began, the animals were allowed to acclimate for three days. During this time, the animals were fed with mice chow (Supplied by ICDDR, B) and water ad libitum. The study was conducted following approval by the Institutional Animal Ethical Committee of Hamdard University Bangladesh.

Oral Glucose tolerance tests for evaluation of glycemic activity

The protocol originally published by Joy and Kuttan [8] was slightly modified in order to conduct the oral glucose tolerance test (OGTT). Mice were divided into six groups of five mice each after they had fasted for twelve hours. The various groups received different treatments like Group 1 received vehicle (1% Tween 20 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (Glibenclamide, 10 mg/kg body weight). Groups 3-6 received, respectively, Methanolic extracts of *Emblica officinalis* fruits (MEEOF) at doses of 50, 100, 200 and 400 mg per kg body weight. The amount of Tween 20 administered was same in both control and experimental mice. Following a period of one hour as described earlier, all mice were orally administered 4 g glucose per kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart following previously published procedures. Blood glucose levels were measured with a glucometer. The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 - We/Wc) \times 100$, where We and Wc represents the blood glucose concentration in glibenclamide or MEEOF administered mice (Groups 2-6), and control mice (Group 1), respectively [9].

Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases [9].

Results

When administered at doses of 50, 100, 200 and 400 mg per kg body weight, methanolic extract of *Emblica officinalis* fruits reduced blood glucose in glucose-loaded mice by 8.0, 20.3, 29.2, and 32.0%, respectively. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, reduced blood glucose levels by 48.8%. The results suggest that Methanolic extracts of *Emblica officinalis* fruits (MEEOF) can be effective in their antihyperglycemic or blood glucose reducing capacities. The results are shown in (Table 1).

Table 1: Lowering action of MEEOF on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	6.70±0.308	-
Glibenclamide	10 mg	3.42±0.216	48.9*
(MEEOF)	50 mg	5.56±0.181	17.0*
(MEEOF)	100 mg	5.08±0.130	34.17*
(MEEOF)	200 mg	4.4±0.122	34.32*
(MEEOF)	400 mg	3.78±0.268	13.58*

All administrations were made orally. Values represented as mean ± SEM (standard error of mean), (n=5); *p<0.05; significant compared to hyperglycemic control animals.

Discussion

Ellagic acid in EO has anti-diabetic properties via stimulating insulin production and reducing glucose intolerance through its impact on the pancreatic β-cells [10]. In this study, the fruits extract of *Emblica officinalis* caused a decreased blood sugar level in oral glucose tolerance test.

Conclusion

The result of this experiment suggest that Methanolic extract of *Emblica officinalis* fruits have glucose lowering capacity to glucose loaded Swiss Albino Mice. Large-scale clinical trials and additional research are required to validate the health benefits of *Emblica officinalis* fruits. Moreover, the richest source of antidiabetic substance is *Emblica officinalis* fruits.

Conflicts of interests

No conflicting interests are stated by the authors.

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