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Original Research Article.

The Blood Glucose Lowering Effect of Aqueous Extract of Emblica Officinalis Fruits in Alloxan Induced Hyperglycemic Rats

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ABSTRACT

Background: Diabetes mellitus which is a chronic metabolic disorder categorized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism.

Objective: In this study our main goal is to evaluate the blood glucose lowering effect of aqueous extract of Emblica officinalis fruit in alloxan induced hyperglycemic rat.

Method: The aqueous extract was orally administered for 4 weeks at a dose of 250mg/kg b.w, 500mg/kg b.w. Weekly estimates of fasting blood glucose level were recorded in normal non-diabetic rats as well as in alloxan induced diabetic rats.

Results: Aqueous extract of Emblica officinalis fruit showed no blood glucose lowering activity in non-diabetic rats. But there was a significant reduction in blood glucose level ($p<0.001$), when compared with diabetic control. Similar results were found when compared with a standard anti-diabetic drug, glibenclamide at a dose of 5mg/kg b.w. Aquouse extract 500mg/b.w produce the maximum response in reducing blood sugar level of diabetic rats.

Conclusion: We can conclude that, the aqueous extracts of E.officinais produce significant change in blood glucose level, specially 500 mg/kg b.w dose on pharmacodynamics response may be useful in insulin resistant cases and to postpone the occurrence of diabetic complications. Further study is needed for better outcome.

Keywords: Diabetes Mellitus, Aqueous Extract, Emblica Officinalis.

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INTRODUCTION

Diabetes mellitus is a major endocrine disorder and growing health problem in most countries. The prevalence of diabetes is increasing worldwide. Epidemiological studies on urbanization and aging influences have shown the prevalence of diabetes for all age group worldwide was estimated to be 2.8 in 2000 & 4.4% in 2030. Bangladesh Institute of Research And Rehabilitation In Diabetes and Endocrine Metabolic Disorders (BIRDEM) carried out a recent survey which revealed the prevalence of the disease in the rural population to be about 6.8%. This type of similarity is also found in most of the under-developed countries.[1,2]

According to WHO (2006) Diabetes is a condition primarily defined by the level of hyperglycaemia giving rise to risk of micro vascular damage (retinopathy, nephropathy and neuropathy) It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (Ischaemic heart disease, stroke and peripheral vascular disease) and diminished quality of life. Diabetic retinopathy, which affects blood vessel formation in the retina of the eye, can lead to visual symptoms including reduced vision and potentially blindness. Diabetic nephropathy, the impact of diabetes on the kidneys, can lead to scarring changes in the tissue, loss of progressively larger amounts of protein in urine, and eventually chronic kidney disease requiring dialysis.[3,4] In diabetes, increased oxidative stress is known to play a decisive role in the pathogenesis of vascular complications. Glutathione depletion may also adversely affect insulin sensitivity, since oxidative stress impairs insulin sensitive glucose transport in adipocytes.

Many synthetic antioxidants have been used in the food industries, but recent publications have mentioned the disadvantages of them and their possible toxic properties for human health. Therefore, natural antioxidant substances are required for the protection against the oxidizing agents. A great number of aromatic, medicinal, spice & other plants contain chemical compounds exhibiting antioxidant properties this includes Garlic (Allium sativum), Ginger (Zingiber officinale), Cinnamon (Cinnamona cassia) etc.[5]

The use of herbal medicines is widespread. Bangladesh is full of medicinal plants, which are used by the people for the treatment of various diseases even at this modern era. There are various medicinal plants in the world, which are the potential sources of the drugs. Traditionally various plants are being used to treat diabetic patients. The conventional pharmacological treatments for type 2 diabetes have a number of limitations, such as adverse effects and high rate of secondary failure. However, medicinal herbs are expected to have a similar degree of efficacy without the troublesome side effects associated with the conventional drug treatment.

One of such compound that has recently been the subject of intense in research is *Emblica officinalis,* commonly known as Amla, is widely distributed in Asia &Africa. Its fruit is commonly used for the treatment of anorexia, constipation, piles, leucorrhea, inflammatory bowls, cough, hemorrhoids, fever, thirst, toxicity of blood & atherosclerosis. This fruit is acrid, cooling, refrigerant, diuretic, laxatives. It is very rich source of vit c & is also used as a medicine to prevent aging due to its strong anti - oxidant property.[6,7]

In this study our main goal is to evaluate the blood glucose lowering effect of aqueous extract of *Emblica officinalis* fruit in alloxan induced hyperglycemic rat.

OBJECTIVES

General Objective

- To assess the blood glucose lowering effect of aquouse extract of *Emblica officinalis* fruit in alloxan induced hyperglycemic rat.

Specific Objective

- To detect the outcome aqueous extract of *Emblica offlcinalis* on fasting blood glucose level of alloxan induced hyperglycaemic rats.
- To evaluate the effect of Alloxan on fasting blood glucose level on rat.

METHODOLOGY

Study Type

This was an Experimental type of study.

Study Place and Period

The study was carried out in the Department of Pharmacology and Therapeutics of Sir Salimullah Medical College in collaboration with Institute of Nutrition & Food Science (INFS) and Department of Pharmaceutical Chemistry of the University of Dhaka from January 2013 to December 2013.

Method

The experiment was carried out with a total number of 36 healthy Swiss Albino rats were purchased from the animal house of Jahangirnagar University, Savar Dhaka. The age of the rat was between 8-10 weeks of both sex and weighing between 100-120 gms. 500gm crushed amla was taken into a conical flask of 5 litre capacity & dissolved in 250ml distilled water & filtered with filter paper., then extract was prepared for use.

It was comprised of 30 rats divided as:

Group A (Non diabetic control Group): In this group rats were given standard rat food and water for 28 days. Fasting blood glucose level was estimated on day 1 and 29th day of the experiment.

Group B *(Emblica officinalis* control group): This group received aqueous extract *of Emblica officinalis* 100 mg/kg b.w orally along with rats food pellets and water for 28 days. Fasting blood glucose level was estimated on day 1 and 29th day of the experiment.

Group C (Diabetic control group): Diabetic control groups were given alloxan 120 mg/kg b.w. (200microlit/rat) intraperitoneally for the induction of diabetes on day 1. After intra peritoneal injection rat were given standard food and water. Fasting blood glucose level was estimated on day 1 (before alloxan), on day 4 (after alloxan to confirm induction of diabetes mellitus) and 29th day of the experiment.

Group D (Experimental group): Experimental groups were divided into different sub groups-D1 & D2.These groups of rats were administered alloxan 120 mg/kg b.w. on first day of the experiment.D1 & D2 were treated with 250 mg & 500mg of aqueous extract of *Emblica officinalis*Then Standard rat food were given that period, They are treated with Group E (Anti diabetic drug group).

Group E (Anti diabetic drug group: This group was given alloxan 120 mg/kg b.w. intraperitoneally on day 1. After alloxan injection rats were given standard food. From day 4 onwards, Glibenclamide orally given at a dose of 5 mg/kg b.w.

Statistical Analysis

The results are given as Mean ± SD for the seven independently performed experiments. Unpaired student's "*t*" test was used to see the level of significance. P value <0.05 was considered statistically significant.ANOVA test was used to see the level of significance among comparison more than two groups, p value < 0.05 was considered statistically significant.

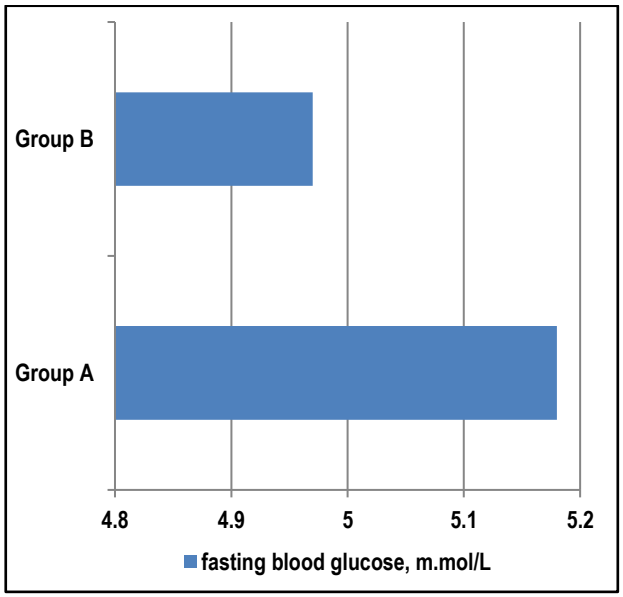

Figure 1: Effect of aqueous extract of *Emblica officinalis* on fasting blood glucose level in non-diabetic rat.

Table 1: Effect of Alloxan on fasting blood glucose level on rat:

Group	No: of Rats	Fasting Blood Glucose (m mol/l) (Mean ± SD) On 29th day of experiment	p-value
A	6	5.18 ±0.14	0.003
C	6	12.35 ± 0.42	

P value is highly significant (0.003)

Table 2: Effect of aqueous extract of *Emblica offlcinalis* on fasting blood glucose level of alloxan induced hyperglycaemic rats.

Group	No: of Rats	Fasting Blood Glucose (m mol/1) (Mean ± SD) On 29th day of experiment	p-value
C	6	12.35±0.42	
D1(250mg/kg b.w)	6	7.12 ±0.12	CVs D_2 < 0.001
D2(500mg/kg b.w)	6	5.75±0.14	CVs D_3 < 0.001

P value is highly significant (<0.001).

Table3: Effect of aqueous extracts of *Emblica officinalis* &Glibenclamide on fasting blood glucose level of alloxan induced hyperglycaemic rats.

Group	Fasting blood glucose level
Group E	6.4± 0.30 m. mol/L
Group D1	7.12 ± 0.12 m. mol/L
Group D2	5.75± 0.14 m. mol/L

RESULTS

In figure-1 shows effect of aqueous extract of *Emblica officinalis* on fasting blood glucose level in non-diabetic rat where in normal control group (Group A), the blood glucose levels (mean ± SD) was 5.18 ± 0.14 m mol/L and in aqueous extract of *Emblica officinalis* treated group (Group B) the serum glucose concentration (mean ± SD) was 4.97 ± 0.08 m.mol/L. The mean reduction in group B compared to that of group A & the result was not statistically significant (p> 0.05). So, aqueous extract of *Emblica officinalis* did not produce any significant change in blood glucose level.

In table-1 shows effect of alloxan on fasting blood glucose level on rats where in normal control group (Group A), the blood glucose levels (mean ± SD) was 5.18 ± 0.14 mmol/L and the serum glucose concentration in Alloxan treated group (Group C) was 12.35 ± 0.42 mmol/L. The mean rise of serum glucose concentration in group C compared to that of group A & the result was statistically significant (p< 0.001). So, Alloxan significantly increase blood glucose level.

In table-2 shows effect of aqueous extract of *Emblica offlcinalis* on fasting blood glucose level of alloxan induced hyperglycemic rats where in group D_2 was treated with aqueous extract 250mg/kg b.w 7.12 ± 012 m. mol/L, in group D_3 was treated with 500mg/kgb.w 5.75± 0.14 m. mol/L and in group C treated with alloxan was 12.35± 0.42 mmol/L .The mean reduction of serum glucose concentration in groupD_2 and D_3 compare to that of group ' C ' & the result was statistically significant (p < 0.001) .So the aqueous extracts of *E.officinais* produce significant change in blood glucose level.

In table-3 shows effect of aqueous extracts of *Emblica officinalis &* Glibenclamide on fasting blood glucose level of alloxan induced hyperglycaemic rats where in group D_2 (aqueous extract 250mg/kgbw) was7.12 ± 0.12 m. mol/L, in group D_3 (treated with aqueous extract 500mg/kgbw) was 5.75± 0.14 m. mol/L and in Glibenclamide treated group (group E) was 6.4± 0.30 m. mol/L.

DISCUSSION

In this study, intraperitoneally (ip) administration of single dose of alloxan (120 mg/kg), increased blood glucose level significantly. The Mean ± SD F.B.G level (m mol/L) of group C on day 4 was 2.58±0.47 and similar observation was reported by number of researchers, who observed the condition of diabetes after 72 hours of intravenous injections of freshly prepared alloxan monohydrate solution at a dose of 120 mg/kg b. w. in male albino rats.[9]

In the present study, the rise in blood glucose level in experimental hyperglycaemic rats were also highly significant as p 0.001.

The mean±SD fasting blood glucose level in Group A was 5.18±0.14 mrnol/L who received laboratory diet for 28 days. Whereas, the mean ±SD fasting blood glucose level in Group B was 4.97±0.08mmol/L, who were treated with aqueous extract of *E.officinalis* 100mg/kg b.w along with lab diet for 28 days to observe the effect *of E.officinalis* on normal rats. There was no significant change (p>0.05) in the mean value of blood glucose level of non-diabetic rat treated with aqueous extract *of E. officinalis*as compared to the normal control.

One study showed that oral administration of *E. officinalis* fruit extracts reduced the blood sugar level in normal & alloxan induced diabetic rat. [10] In this study non diabetic control group treated with aqueous extract shows non-significant (p>0.05) change.

The serum glucose concentrations (mean±SD) in alloxan treated group (Group-C) was 12.35 ± 0.42 mmol/L & in the control group (Group-A) this was 5.18 ± 0.14mmol/L. The difference was statistically significant (p<0.001). Hence, alloxan-treatment has significantly raised the serum glucose levels in rats.

The dose of aqueous extract of *E officinalis* (250mg/kg b.w) used in this study was chosen based on the dose used in the research done by one study & the duration of the study (28 days or 4 weeks) was selected according to one study.[11]

Different study suggested that higher amount doses can be responsible for reduction blood glucose level in diabetic rats.[10] In the study we found similar types of result where administration of aqueous extract of *E. officinalis* 250mg/kg & 500mg/kg b.w D_1 & D_2 group produced a significant ($p<0.001$) reduction in blood glucose level as compared to diabetic control (group C) and it was observed that 500smg/kg b.w reduced higher amount of blood glucose level than 250mg/kg.

Some earlier report suggested that few species of *Emblica qfficinalis* were found to involve in regeneration & rejuvenation of beta cells leading to an increased insulin production &secretion.[12]

Another study described that some of the important constituents of *Emblica qfficinalis* (including gallic acid, gallotanin, ellagic acid & corilagin) possess anti-diabetic effect through their antioxidant & free radical scavenging properties.[13]

In the present study, administration of Glibenclamide at 5mg/kgb.w in Group -E produced highly significant ($p<0.001$) reduction in blood glucose level in alloxan induced hyperglycemic rats .A similar observation was made by one report ,where Glibenclamide at 5mg/kg.b.w was used in alloxan induced hyperglycemic rats & blood glucose level reduced significantly($p<0.001$).[14]

LIMITATION

Small sample size and duration of time is short.

CONCLUSION

From our result we can conclude that additive action of aqueous extract of *E officinalis* specially 500 mg/kg b.w dose on pharmacodynamics response may be useful in insulin resistant cases and to postpone the occurrence of diabetic complications. Further study is needed for better outcome.

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