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Research Article

Effect of *Emblica officinalis* Fruit Extract on Gluconeogenesis in Allaxon Induced Diabetic Mice

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ABSTRACT
Emblica officinalis (Amla), known as Indian gooseberry, has been used extensively in the ancient Indian Ayurveda as a potent rasayana i.e. a herbal formulation that helps attain longevity and rejuvenation. Administration of *Emblica officinalis* fruit extract to allaxon induced diabetic mice showed increase in the contents of pyruvate and lactate in liver, kidney and thigh muscle tissue. It also showed decreased levels of glucose - 6 - phosphatase indicating reduced gluconeogenesis. Gluconeogenesis from lactate, pyruvate, glycerol and amino acids is a major essential function of glucose for nervous system at times when dietary carbohydrates is lacking. The lactate levels in all the tissues have shown a fall in the diabetic state and after treatment with the fruit extract have shown an increase indicating the reversal towards normalcy.

Keywords: *Emblica officinalis*, Diabetic mice, Fruit extract, Pyruvate, Lactate, Glucose-6-phosphatase, Gluconeogensis.

INTRODUCTION

Emblica officinalis (Family Euphorbiaceae) is a medicinal plant described in Ayurveda, which is the oldest medicinal system in the world and the World Health Organization has approved its efficacy [(1)]. The fruit (Indian gooseberry) is used as a major constituent in several Ayurvedic preparations such as Chyavanprash, Brahma Rasayan, Amalak, Haritaki, Triphala and Septilin, which promote health and longevity[(2)]. Triphala is a traditional Ayurvedic herbal formulation which is considered an important rasayana in Ayurvedic medicine [(3)]. *Emblica officinalis* extract has been shown to possess high antioxidative, anticancer, lipid-lowering, antisclerotic, hepatoprotective, and anti-HIV potentials [(4,5,6)].

Diabetes mellitus is a major global health concern. In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double [(7)]. In traditional practice, medicinal plants are used in many countries to control diabetes mellitus. The hypoglycemic action of these medicinal plants is being studied extensively [(8)]. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones [(9)]

Due to the excretion of sugar in urine during diabetes, the animal derives glucose from non-carbohydrate sources like pyruvate, lactate, amino acids etc, and therefore the activity of gluconeogenic enzymes increases under such condition [(10)]. Glucose-6-phosphatase occupies a unique metabolic position catalyzing the terminal reaction in gluconeogenesis, the transformation reaction of glucose-6-phosphate into glucose [(11)]. During present study, the amounts of these metabolites were estimated in diabetes induced mice and in diabetes induced mice treated with the fruit extract of *Emblica officinalis.*

MATERIAL AND METHODS

Source of plant extract: The fruit extract of *Emblica officinalis* obtained from Heritage Bio Natural Products Pvt Ltd, Uppal, Hyderabad, Telangana State and India.

Preparation of the extract: Take the cleaned fruit material and crush material extract with alcohol of graded strength and concentrated liquid (Volume reduction under vacuum) and purified material liquid extract (Apporx.30% solid) will be formed which is converted into thick semi solid extract. The extract was suspended uniformly in distilled water and injected intraperitonially.

Induction of Alloxan Diabetes: Alloxan Monohydrate of 100mg/Kg body Weight was dissolved in distilled water [(12)] and administered using a micro syring to overnight fasted mice. The route of administration was decided based on the literature [(13,14)].

Experimental set up for evaluation of hypoglycemic activity of the extract in diabatised mice: Three groups of animals 25-30 gm were maintained each comprising of 6 male mice of same age for experimentation. The first group was maintained as control, the second and third groups were diabetised with a dose 100mg/kg body weight of alloxan (IP) and after a time interval of 48 hours blood glucose levels were estimated to confirm the induction of diabetes and glucose tolerance test was performed. The

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Table 1: The levels of pyruvate in liver, kidney and muscle of control, diabetic and diabetic treated with fruit extract of *Emblica officinalis*

Tissue		Control	Diabetic	Diabetic treated with extract
Liver	Mean± SE	9.80±0.13	8.50±0.07	10.00±0.14
	% variation		12.89%	1.52%
Kidney	Mean± SE	5.31±0.09	4.31±0.10	5.50±0.07
	% variation		18.67%	3.70%
Muscle	Mean± SE	7.26±0.08	6.73±0.06	7.350.11
	% variation		7.71%	1.23%

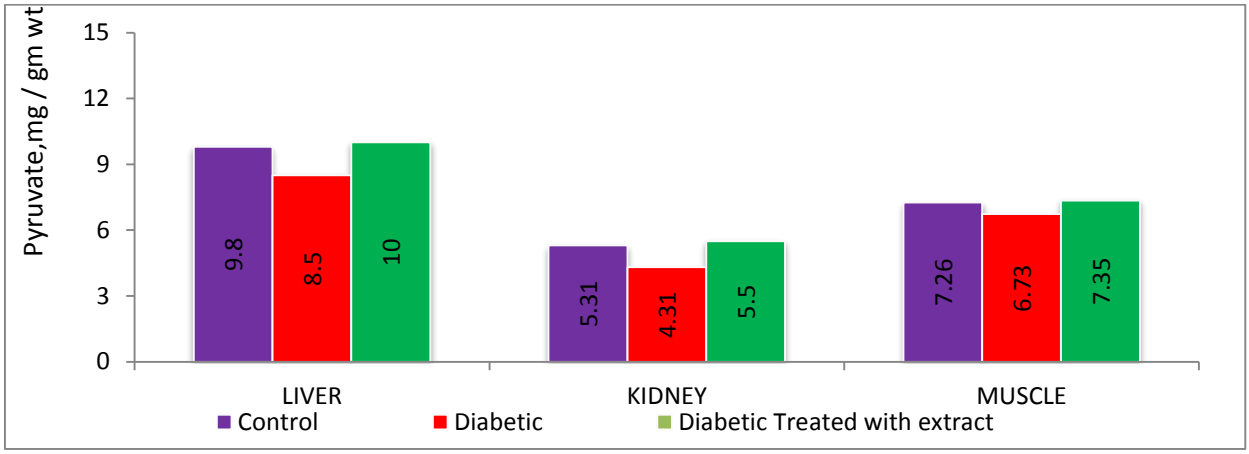

Fig 1: The levels of pyruvate in liver, kidney and muscle of control, diabetic and diabetic treated with fruit extract of Emblica officinalis.

third group was administered with *E. officinalis* fruit extract of a dose of 600mg/kg body weight intra pertonially. 3hours after administration of the extract to the third group, a slice of liver and kidney and thigh muscle were collected from all the mice of all three groups for estimation of Pyruvate, Lactate and glucose-6-phosphatase.

Pyruvate content was estimated following the method [15], while lactic acid was estimated by the method [16]. Activity of Glucose-6-phosphatase (G6pase-ec 3.1.3.9) was assayed by the method [17].

RESULTS AND DISCUSSION

Pyruvate: The pyruvate content in liver in diabetic state reduced by 12.89%, while in diabetic treated animals it increased by 1.52%, as compared to that in control. The pyruvate content in kidney also decreased by18.67% in diabetes induced animals and the animals treated with *Emblica officinalis* fruit extract showed slight increase in pyruvate content. The pyruvate in muscle tissue of allaxon diabetic animal showed a reduction by 7.71% compared with controls, while the diabetic treated with extract showed an increase of 1.23% above the normal indicating recuperation of animal from the diabetic state.

Pyruvate in diabetic condition: The pyruvate content in the tissues, liver, kidney and muscle depleted in the diabetic state in the present study and a significant increase in the tissue of diabetic animal treated with the fruit of extract of *Emblica officinalis*. This indicates its utilization which may be due to an enhanced gluconeogenesis occurring during diabetes as a result of insulin deficiency. Ashmore[18] has demonstrated that there is an enhanced formation of glucose from carbohydrate metabolic intermediates i.e. the glucose-6-phosphate and pyruvate in diabetes.

Pyruvate in diabetic treated condition: The return of pyruvate to the control levels in the diabetic animal treated with the plant extract indicates the effect of the extract in the treatment of diabetes. In present study on the treatment with the extract, the decreased activity levels of glucose-6-phosphatase denotes the non-occurrence of gluconeogenesis and hence a decrease in glucose from its level in diabetic state.

Lactate: The levels of lactate show a fall by 17.13% in liver tissue of diabetic compared with control, while the diabetic animals treated with extract showed only 1.60% less than the control. There was a decrease of 18.59% in kidney tissue of alloxan diabetic mice while there was a fall of 4.56% from the controls in diabetic treated with the fruit extract of *Emblica officinalis*. The lactate levels in muscle tissue decreased by 14.43% in diabetic mice compared with the controls, while the diabetic treated with extract the lactate levels increased by 0.56%. The lactate levels in all the tissues have shown a fall in the diabetic state and after treatment with the fruit extract have shown an increase indicating the reversal towards normalcy.

Lactate in diabetic state: The lactate in the present study significantly decreased in diabetic state, compared with the control levels. This depletion in the lactate levels could be due to its utilization as gluconeogenic precursor since; during diabetes there is a hampered glucose uptake by the tissues. Gluconeogenesis from lactate, pyruvate, glycerol and amino acids is a major essential function of glucose

Table 2: The levels of lactate in liver, kidney and muscle tissue of control, allaxon diabetic and diabetic treated with the fruit extract of *Emblica officinalis*.

Tissue		Control	Diabetic	Diabetic treated with extract
Liver	Mean± SE	17.83±0.20	14.75±0.23	17.50±0.27
	% variation		17.13%	1.68%
Kidney	Mean± SE	14.25±0.18	11.50±0.34	13.64±0.22
	% variation		18.59%	4.56%
Muscle	Mean± SE	17.66±0.18	15.16±0.30	17.51±0.21
	% variation		14.43%	0.56%

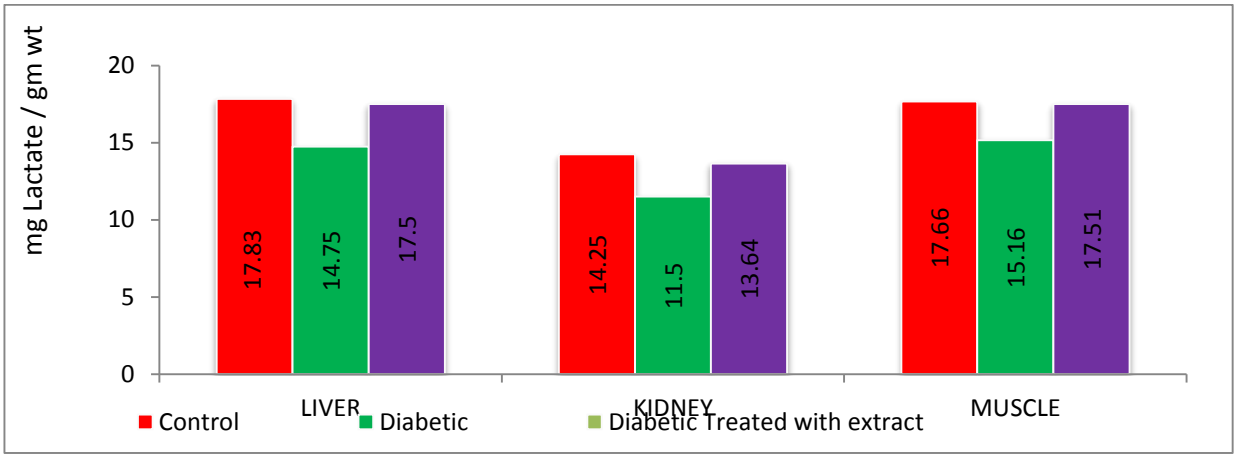

Fig 2: The levels of lactate in liver, kidney and muscle tissue of control, allaxon diabetic and diabetic treated with the fruit extract of Emblica officinalis.

for nervous system at times when dietary carbohydrates is lacking. It was observed [19] that glucose was the major product of lactate, pyruvate and fructose metabolism.

Thus, depleted levels of lactate during the diabetic state clearly show the hepatic gluconeogenesis from this metabolite.

Apart from the liver, the lactate is metabolised in kidney tissue also for conversion into glucose as kidney is also a gluconeogenic organ [20]. The reduction of lactate levels in muscle might have been brought about by the rapid removal of this metabolite for the enhancement of gluconeogenesis in liver to meet the energy requirement. The lactate utilization by skeleton muscles in mammals for glycogen synthesis is reported [21].

Lactate in diabetic mice treated with extract: The reversal in lactate levels due to decrease in hepatic glucose production indicating a decrease in the rate of occurrence of gluconeogenesis and thus non-utilization of gluconeogenic precursor one of which is lactate suggesting that the extract like other hypoglycemic agents might have suppressed the hepatic glucose output reducing the gluconeogenesis.

Glucose-6-phosphatase: The glucose-6-phosphatase levels in the diabetic mice showed an increase of 36.85% compared with controls while the diabetic treated with extract have shown a variance of 16% indicating a decreasing trend towards normalcy.

Glucose-6-phosphatase in diabetic state: In the present investigation the glucose-6-phosphatase levels have increased significantly in allaxon diabetic mice indicating an enhanced gluconeogenesis during diabetes. Ashmore[22] has demonstrated a marked elevation in glucose-6-phosphatase activity in allaxon diabetic rats and also reported a significant increase in glucose-6-phosphatase in diabetic rats[14].

In diabetes, hyperglycemia occurs as a result of impaired transport and uptake of glucose into muscle and adipose tissue. Repression of the key glycolytic enzymes and depression of gluconeogenic enzymes promotes gluconeogenesis in the liver, which further contributes to hyperglycemia.

Glucose-6-phosphatase in diabetic mice treated with extract: The activity levels of glucose-6-phosphatase in diabetic mice treated with fruit extract in the present study showed a significant fall almost to the levels of enzyme in controls indicating decreased gluconeogenesis. This observation suggest that the fruit extract could have led to the stimulation of beta cells of pancreas resulting in increased secretion of insulin thus leading to decreased gluconeogenesis.

CONCLUSION

The increase in pyruvate and lactate levels and decrease in the levels of glucose-6-phosphate indicates reduced gluconeogenesis after administration of fruit extract of *Emblica officinalis* to the allaxon diabetic mice. The depletion of lactate could be due to its utilization in the formation of glycogen and in turn glycogen could have been utilized for energy purpose in diabetic condition or lactate could have also been directly utilized as gluconeogenic precursor.

Table 3: The levels of glucose-6-phosphatese in control, allaxon diabetic and diabetic treated with the fruit extract of *Emblica officinalis*.

Tissue		Control	Diabetic	Diabetic treated with extract
G6pase	Mean± SE % variation	7.435±0.021	10.175±0.044 36.85%	7.643±0.044 20.85%

Note: - values are significant at P<0.05

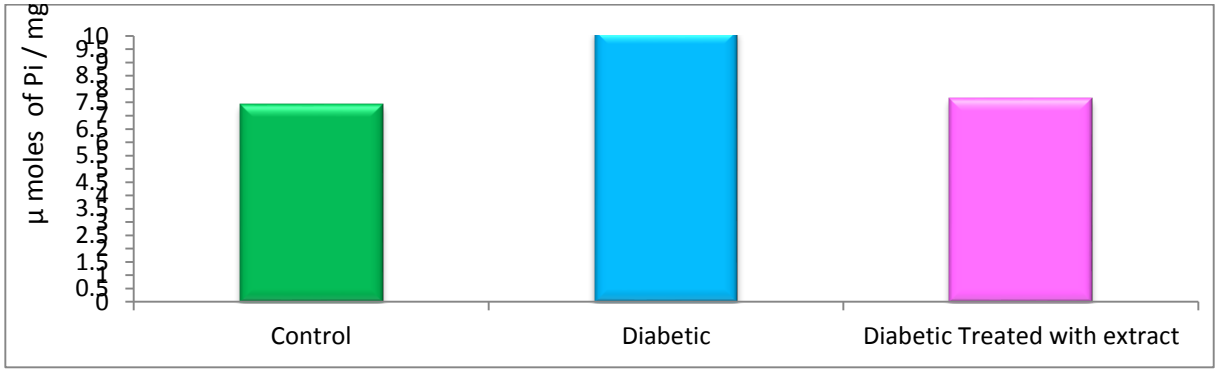

Fig 3: Activity levels of glucose-6-phosphatese in control, diabetic and diabetic treated mice with fruit extract of Emblica officinalis

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