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The Effect of *Phyllantus emblica* Linn on Type - II Diabetes, Triglycerides and Liver - Specific Enzyme

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Abstract: The effect of aqueous fruit extract of *Phyllanthus emblica* Linn was studied on type-II diabetes, triglycerides (TG) and liver-specific enzyme, alanine transaminase (ALT). Our study showed that aqueous fruit extract, in a dose of 200mg/kg body weight, significantly decreased the blood glucose level after its intra-peritoneal administration in alloxan-induced diabetic rats ($p<0.05$). Almost similar decreased in glucose level was also observed by chlorpropamide, a known antidiabetic drug in a dose of 84 mg/kg. The aqueous extract also induced hypotriglyceridemia by decreasing TG levels at 0, 1, 2 and 4 hours in diabetic rats ($p<0.05$). In addition, the extract was also found to improve liver function by normalizing the activity of liver-specific enzyme alanine transaminase (ALT).

Key words: Type-II diabetes, cholesterol, triglycerides, alanine transaminase, aqueous fruit extract of *P. emblica*

Introduction

The incidence of diabetes is considered to be high worldwide (ADA, 2000). According to the International Diabetes Federation, there are 246 million people with diabetes on the globe and this figure will rise to 380 million by the year 2025 (Fatima, 2007). Diabetes is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production (type-I) or insufficient insulin utilization (type-II) (Marshal and Bangert, 2004). Of these, type-II (NIDDM; Non-Insulin - Dependent - Diabetes Mellitus; Adult on set) diabetes is the major problem of today and it accounts for nearly 95% of total diabetic population (Mycek *et al*., 2000). The commonly practiced treatments of diabetes include oral antidiabetic drugs, insulin injections (in severe cases) and management through diet and physical exercise (Vats *et al*., 2002). Beside these, people especially in Asian countries like Pakistan, still use extracts of different medicinal plants with or without the advice of Hakims or Ayurvedic practitioners. Few of these medicinal plants have been scientifically investigated. *Phyllanthus emblica* Linn, a medicinal plant used in diabetic therapy, was selected for the present study.

Phyllanthus emblica (*Emblica officinals* Gaertn; family: Euphorbiaceae) Linn, commonly known as Amla, is widely distributed in Asia and Africa (Rao and Siddiqui, 1964). Its fruit is commonly used for the treatment of anorexia, constipation, piles, leucorrhea, inflammatory bowls, cough, hemorrhoids, fever, thirst, toxicity of blood and atherosclerosis (Thakar and Mandal, 1984). This fruit is acrid, cooling, refrigerant, diuretic, laxatives. It is a very rich source of vitamin C and is also used as a medicine to prevent aging (rejuvenation) due to its strong antioxidant properties (Sairam *et al*., 2002). The present investigation was designed to determine the effect of aqueous fruit extract of *P. emblica* Linn on type-II diabetes and triglycerides (TG) since changes in lipid metabolism are secondary to diabetes. In addition, special attention was also given to liver-specific enzyme alanine transaminase (ALT).

Materials and Methods

Animals: Male albino rats, weighing 150-200 grams, were purchased from Agha Khan University Hospital, Karachi and were kept under usual management conditions in conventional animal house. They were given standard laboratory diet with access to water *ad libitum*.

Alloxan tetrahydrate (sigma): It was used to induce diabetes in a dose of 120 mg/kg body weight for 3 consecutive days in rats intra-peritoneally (Qureshi and Hasnain, 1997).

Chlorpropamide (sigma): It was used as positive control in a dose of 84 mg/kg body weight (Kamanyi *et al*., 1994).

Reagent kits for chemical analyses (human): For the estimation of blood glucose (GOD-PAP method), triglycerides (GPO-PAP method) and alanine transaminase (ALT), reagent kits were supplied by Human, USA.

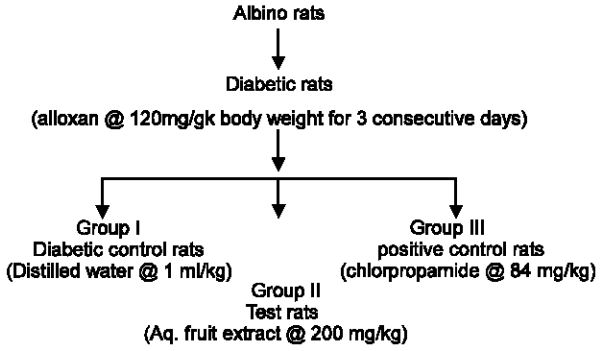

Fig. 1: Animal grouping on the basis of treatments.

Plant material: Fruits of *P. emblica* were purchased from Hamdard Dawakana, Sadar, Karachi and identified by experts in Botany department, University of Karachi, Karachi-75270, Pakistan.

Preparation of extract: 40 grams grinded powder of fruits of *P. emblica* was extracted with 1L boiling water for 30 minutes with magnetic stirrer. The heated decoction was obtained and allowed to cool at room temperature. The decoction was filtered through Whatman No. 1 filter paper twice. The filtrate was then lyophilized. Finally brown colored residue was collected and used in a dose of 200mg/kg body weight to investigate its effects on desired biochemical parameters (Qureshi and Hasnain, 1997).

Experimental procedure: Experimental rats were made diabetic with help of alloxan tetrahydrate and divided into 3 groups according to the treatments (Fig. 1). All rats were kept on overnight fast (12-14 hours) before starting the experiment. After each treatment, rats were sacrificed at intervals of 0, 1, 2 and 4 hours. Serum was separated from collected blood and used to analyze desired biochemical parameters on Spectro UV-Visible Auto, PC Scanning Spectrophotometer, Labomed, Inc.

Statistical analysis: Results were recorded and expressed as mean ± SEM. The data were analyzed by paired *t*-test (Graphpad prism Version 4.0). Differences of groups II and III with diabetic control (group-I) were considered as statistically significant when $p < 0.05$.

Results

Effects of aqueous extract and chlorpropamide on glucose level (mg/dl): The glucose concentrations (mg/dl) in diabetic control rats were 223.3 ± 7.6, 208.3 ± 6, 209 ± 5.8 and 278.6 ± 7.6 respectively at 0, 1, 2 and 4 hours. Where as, there was a significant ($p<0.05$) fall observed in glucose levels of diabetic rats treated with aqueous extract of *P.emblica* @ 200 mg/kg body weight as 106±2.8, 92±4.5, 90.6±8.7 and 179±6.4 at 0, 1, 2 and 4 hours respectively. Similarly, diabetic rats treated with

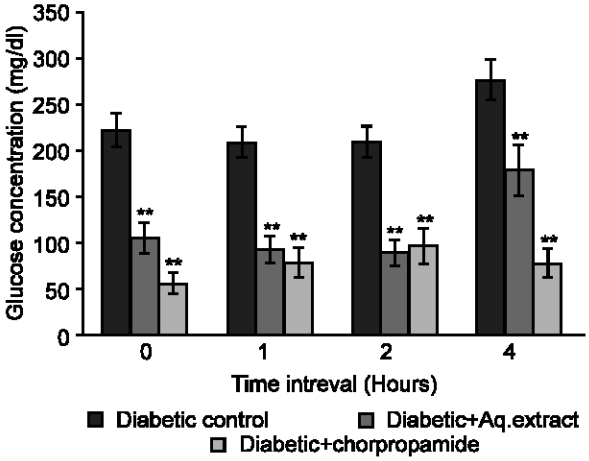

Fig. 2: Changes in glucose concentration at different time intervals in alloxan-induced diabetic rats, diabetic rats treated with aqueous fruit extract and diabetic rats treated with chlorpropamide. Each bar represents mean ±SEM (n=6). **Represents significant difference from respective diabetic control ($p < 0.05$).

chlorpropamide @ 84 mg/kg body weight also showed a decreased level of glucose ($p<0.05$) as 55.6±2.9, 80±5.7, 95±2.8 and 77.2±1 at 0, 1, 2 and 4 hours respectively (Fig. 2).

Effects of aqueous extract and chlorpropamide on TG levels (mg/dl): A significant difference ($p<0.05$) was found on TG by observing a decrease in its level in diabetic rats treated with aqueous extract and chlorpropamide at 0, 1, 2 and 4 hours as compared to diabetic control rats (Fig. 3).

Effects of aqueous extract and chlorpropamide on ALT activity (U/l): Diabetic rats treated with aqueous extract showed a significant difference ($p<0.05$) in ALT activity as compared to diabetic control rats by bringing the enzyme activity in its normal level as 26.4±3.6, 33.5±0.7, 22.8±0.7, 29.2±2.5 respectively at 0, 1, 2 and 4 hours. Where as its high activities were observed as 42.8 ± 6.1, 48.5±0.7, 41±2.5 and 33.5±9.4 respectively at 0, 1, 2 and 4 hours in diabetic control rats. However, chlorpropamide did not show any significant effect on ALT level of diabetic rats (Fig. 4).

Discussion

Type II diabetes is one of the major health problems throughout the world especially in adults of age above 35 years in both sexes (Marshal and Bangert, 2004). In spite of the presence of number of synthetic oral antidiabetic drugs in the market, researchers are now diverted their attention to different herbs and medicinal plants in order to find out new active principle with less

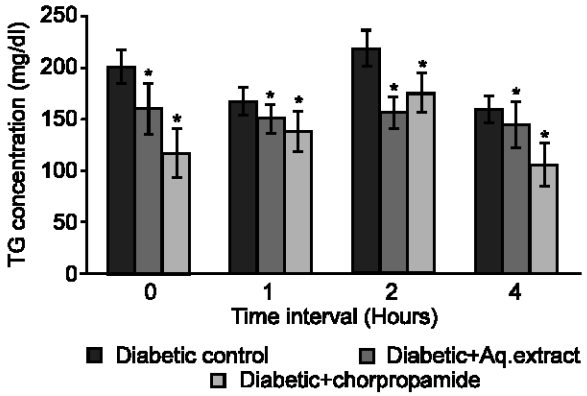

Fig. 3: Changes in triglycerides (TG) concentration at different time intervals in alloxan-induced diabetic rats, diabetic rats treated with aqueous fruit extract and diabetic rats treated with chlorpropamide. Each bar represents mean ±SEM (n= 6). *Represents significant difference from respective diabetic control (p < 0.05).

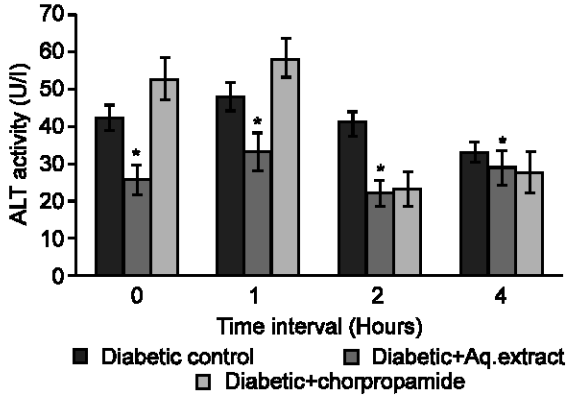

Fig. 4: Changes in alanine transaminase (ALT) activity at different time intervals in alloxan-induced diabetic rats, diabetic rats treated with aqueous fruit extract and diabetic rats treated with chlorpropamide. Each bar represents mean ±SEM (n= 6). *Represents significant difference from respective diabetic control (p < 0.05).

side effects and better antidiabetic activity (Beigh *et al.*, 2002). Therefore, *P. emblica* (Amla) Linn was selected for the present study in order to provide some help in patronizing indigenous drugs.

According to the obtained results, it has been confirmed that aqueous fruit extract of *P. emblica* has a potent antidiabetic activity by showing a significant fall ($p<0.05$) in blood glucose level of diabetic rats treated with aqueous fruit extract @ 200 mg/kg body weight. Maximum decrease in blood glucose level was observed after 1 and 2 hours of treatment. As diabetes was induced by alloxan in experimental rats (Qureshi and Hasnain, 1997), the antidiabetic effect of aqueous fruit extract might be extra-pancreatic either by inhibiting glycogenolysis, hepatic gluconeogenesis and glucose absorption from intestine or by increasing glucose absorption in cells of peripheral tissues (muscles and adipose tissues) and hepatic glycogenesis (Kamanyi *et al.*, 1994). However it was very interesting that almost same antidiabetic effect was observed by chlorpropamide, which is known to produce its effect by stimulating the release of endogenous insulin (Kamanyi *et al.*, 1994), this suggests that may be alloxan @ 120 mg/kg was not sufficient for complete destruction of ß - cells and few cells were remain have capability to regenerate. This finding supports the earlier reports of few *Phyllanthus* species, which were found to involve in regeneration and rejuvenation of ß - cells leading to an increased insulin production and secretion (Daisy *et al.*, 2004).

Abnormalities to lipid metabolism may be secondary to diabetes and it was also confirmed in alloxan-induced diabetic rats, where an increase in plasma glucose level was accompanied by an increase in TG levels. In our study, both aqueous fruit extract and chloropropamide were found to decrease TG level at 0, 1, 2 and 4 hours with a significant difference as compared to diabetic control ($p<0.05$). The significant effect of aqueous extract on diabetic hypertriglyceridemia could be due to its effects on glycemic control. Glycemic control is the major determinant of total and very low density lipoproptein (VLDL) triglyceride concentrations (Markku, 1995). The improved glycemic control by sulphonylureas accompanied with decrease VLDL and total triglyceride levels was previously reported by Rao *et al.*, 1999. The same hypotriglyceridemic activity of chlorpropamide was also observed in the present study. Many medicinal plants having hyoptriglyceridemic effect in diabetic animals were reported such as *Momordica charantia* (Kedar and Chakrabarti, 1982).

Beside antidiabetic and hypotriglyceridemic effects, the aqueous extract was also found to improve liver function by normalizing the activity of liver-specific enzyme ALT, ranging from 22.8-33.5 U/l ($p<0.05$) as compared to diabetic control rats, where high activities of ALT were found, ranging from 26.5-84 U/l. This finding was also supports the hepatoprotective property of Amla (Gulati *et al.*, 1995).

Conclusion: The present study demonstrates that aqueous fruit extract of *P. emblica* (Amla) Linn has significant antidiabetic and hypotriglyceridemic activities. In addition, it did not show any toxic effect and found to improve the liver function. Therefore this plant could be selected for further work to isolate active principle having antidiabetic activity with less side effects.

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