RESEARCH PAPER

Effects of *Emblica officinalis* commercial formulation in Type 2 Diabetic Patients

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The levels of blood glucose and malondialdehyde in type 2 diabetic patients have been studied after the administration of *amalaki* capsules containing 250 mg fruit powder of *Emblica officinalis* twice a day for 60 d. It was observed that the drug treatment decreases blood glucose levels from 203±82 mg/dl (initial) to 189±55.2 mg/dl (after 60 d of treatment). However, the decrease was statistically insignificant ($p>0.05$). On the other hand, administration of amalaki for the same period of time resulted in a significant decrease in malondialdehyde levels from 7.55±2.3 nM (initial) to 5.36±0.8 nM (after 60 d of treatment). The decrease in the levels of the malondialdehyde was statistically significant ($p<0.05$). In the present study, no direct relationship was observed between blood glucose levels and malondialdehyde levels (lipid peroxide levels) of type 2 diabetic patients.

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia. The metabolic disturbance involves alteration in the metabolism of fats, proteins and carbohydrates, reflecting a state of insulin deprivation. This occurs due to deficient insulin secretion or due to factors opposing the tissue effects of insulin or both[1]. Free radicals have been implicated as one of the major causes of diabetic complications. The increase in free radical levels in diabetic patients leads to oxidative stress and the complications arising form it. In type 2 Diabetes mellitus, oxidative damage has been blamed for the development of the disease as well as for its various accompanied complications. Oxidants inhibit glucose metabolism in the glycolytic pathway and at the level of oxidative phoshorylation, thereby causing a sugar overload in the blood. Increased blood sugar levels cause autoxidation of glucose and glycation of proteins, which are implicated in diabetic complications[2].

Oxidative stress can be evaluated by using different kinds of markers, of which lipid peroxidation is one[2]. Lipid peroxidation is the peroxidative breakdown of unsaturated

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fatty acids following an oxidative stress. Lipid peroxidation is a free radical process. It is one of the basic mechanisms involved in reversible and irreversible cell and tissue damage. It leads to degradation of lipid membrane with intra and extracellular targets and also to the production of new reactive oxygen species[3]. The free radicals thus produced attack cell structures within the body. The prime targets of reactive oxygen species attack are polyunsaturated fatty acids present in the membrane lipids causing lipid peroxidation, which may lead to disorganization of cell structure and function. Degradation of peroxidised lipids yields a wide variety of end products, including malondialdehyde[4].

Antioxidants are the substances that prevent or slow down the oxidation reactions. Antioxidants stop the free radical generation by trapping the free radicals, by providing the missing electron to the free radicals and thus they inhibit the chain reactions, which can lead to destruction of healthy cells. Several medicinal plants are known to possess antioxidant property[5]. One of these plants is *Emblica officinalis* whose crude drug powder is used in Indian traditional system of medicine (Ayurveda) for the treatment of pancreas related disorders[6]. Previous investigations of the extracts of *E. officinalis* revealed that they possess

antioxidant activity[7,8], which may be due to the presence of high levels of superoxide dismutase[9]. The extracts have also been reported to have hypolipidemic and hypoglycaemic[10,11] as well as antimutagenic activities[12].

In the present study, *amalaki* capsules containing 250 mg of crude fruit powder of *E. officinalis* have been evaluated for their effects on blood glucose and malondialdehyde levels in type 2 diabetic patients with an aim of finding out whether there is any relationship between blood glucose and malondialdehyde levels in such patients.

MATERIALS AND METHODS

Thiobarbituric acid GR (TBA)was purchased from Loba Chemie, Mumbai and trichloroacetic acid (TCA) was procured from Qualigens, Mumbai. 1,1,3,3-Tetra-ethoxy propane (TEP) was a product of Sigma-Aldrich, USA. Hydrochloric acid and chloroform were purchased from S. D. Fine Chemicals, Mumbai. Methanol was procured from E. Merck India Ltd., Mumbai and *amalaki* Capsules of Himalaya Drug Company, Bangalore were used as received.

Subjects:

Statement of purpose of the study along with informed consents from the persons willing to participate in the present study was submitted to the Ethical Committee of Kakatiya University, Warangal. After proper scrutiny the Ethical Committee has approved and granted permission to carryout this work. A total number of 16 volunteers were chosen for the present study and they were divided in to two groups. The first group comprised of 10 diabetic patients and the second group 6 healthy volunteers. The diabetic subjects were of the 40-60 y age group while healthy volunteers were of the 25-35 y age group. The healthy volunteers were chosen to assess changes in free radical levels that occur in general.

The patients were treated with amalaki capsules for 60 d. On the day 1, blood glucose and serum malondialdehyde levels were determined in all test subjects. Each patient was provided with amalaki capsules sufficient for 60 d and was instructed to take two capsules per day, one after breakfast and another after dinner. Blood samples were collected once every 15 d and the blood glucose and malondialdehyde levels were determined.

Collection and processing of blood samples:

Blood (5 ml) was collected from the median cubital vein of the volunteers with the help of sterile disposable syringes, transferred into clean, dry, labeled 10 ml test tubes and capped tightly. The collected blood samples were then centrifuged in a refrigerated centrifuge at 3000 rpm for about 30 min. Serum from the test tubes was withdrawn with the help of a micropipette and transferred into 2 ml labeled Eppendorff tubes. The serum samples were stored at −20° till analyzed.

Measurement of Blood glucose levels:

The blood glucose levels were measured using a Glucometer (Gluco Men Glyco, A. Menarini Diagnostics, Italy). The Glucometer measures the blood glucose levels by the following mechanism. First a Gluco Men Sensor (glucose strip) was inserted into the Glucometer; later on a drop of blood is placed on the target area of the strip, which then by capillary action will flow into the reaction cell where the enzyme glucose oxidase converts blood glucose to gluconolactone. A mediator present in the cell then transfers the electrons generated in the reaction process from the enzyme to conductors. The meter measures this reaction as a small electrical current and then calculates the corresponding glucose level.

Determination of Malondialdehyde levels:

Malondialdehyde levels were determined using the thiobarbituric acid reactive substances (TBARS) Method[13]. The thiobarbituric acid (TBA) assay is one of the most widely used methods to determine the extent of lipid peroxidation in serum samples. A standard curve was plotted using 1,1,3,3-tetra-ethoxy propane (TEP) as a standard. Solutions (0.5.ml) of 5 to 60 nM TEP were taken in test tubes and to them 0.5 ml of 30% trichloroacetic acid solution was added. Then 100 μl of 1% thiobarbituric acid solution was added using a micropipette into each test tube. All the test tubes were covered with aluminium foil and then heated at 95° for 1 h. The samples were then cooled in an ice bath for 10 min and then the absorbance of the solutions was read at 540nm. A standard curve of absorbance versus concentration was made.

Analysis of serum samples:

Serum samples (0.5ml) were transferred into clean, dry and labeled 10 ml test tubes. To these test tubes 0.5 ml of 30% trichloroacetic acid solution was added and mixed thoroughly. Then 100 μl of 1% thiobarbituric acid solution was added and all the test tubes were covered with aluminium foil and heated on a water bath at 95° for 1 h. The test tubes were cooled in an ice bath for about 10 min and centrifuged at 3000 rpm for about 10-15 min. The clear

supernatants (50 μl) were then transferred into clean dry, labeled test tubes with the help of micropipette and diluted with 2 ml of distilled water. The absorbances of the resulting solutions (2.5 ml) were read at 540 nm using a spectrophotometer (Systronics, Hyderabad). Based on the absorbance values obtained, the concentration of malondialdehyde in the serum samples was calculated using the standard curve.

RESULTS AND DISCUSSION

The effect of amalaki capsules on the blood glucose levels and malondialdehyde levels was studied in type 2 diabetic patients. The fig. 1 shows the effect of amalaki capsules on blood glucose levels of diabetic patients. The administration of 250 mg amalaki capsule twice a day for 60 d, decreased blood glucose levels from 203±82 to 189±55 mg/dl. However, the decrease was statistically insignificant (p>0.05). The malondialdehyde levels, which are an indication of extent of lipid peroxidation process, were also determined in this study before and after treatment with amalaki capsules and the results are depicted in fig. 2. The results indicate that there was a decrease in malondialdehyde levels from 7.55±2.28 to 5.36±0.80 nM, which was statistically significant (p<0.05).

The decrease in malondialdehyde levels in type 2 diabetic patients after administration of amalaki capsules

is an indication that the drug, whose sole active ingredient is E. officinalis inhibits lipid peroxidation, resulting in reduced levels of free radicals. Amla is one of the famous Indian fruits. It is rich source of vitamin C and its ascorbic acid content is next to only that of Barbados cherry, Malphigia glabra. Amla is also one of the three constituents of the famous ayurvedic preparation, Triphala, which is prescribed, in digestive disorders[14]. It has been reported that the vitamin C present in the Amla is stable to heat and also to storage conditions[15]. Amalaki capsules are also known to contain high amount of hydrolysable tannins such as gallic acid and ellagic acid[9]. The tannoid principles together with vitamin C are considered to be responsible for the antioxidant activity of the drug in diabetic patients by decreasing the malondialdehyde levels. The antioxidant activity of amalaki capsules can be attributed to the phenolic groups present in gallic acid and ellagic acid. These phenolic groups undergo keto-enol tautomerism, thus donating a hydrogen atom, which can stop the free radical chain reaction at the initial stages itself. The vitamin C present in the capsules acts as an antioxidant by sequestering the metallic ions, which are active pro-oxidants in free radical chain reactions.

From the results obtained in the present work, it can be concluded that administration of Amalaki capsules (which contains the fruit powder of Emblica officinalis) to type 2
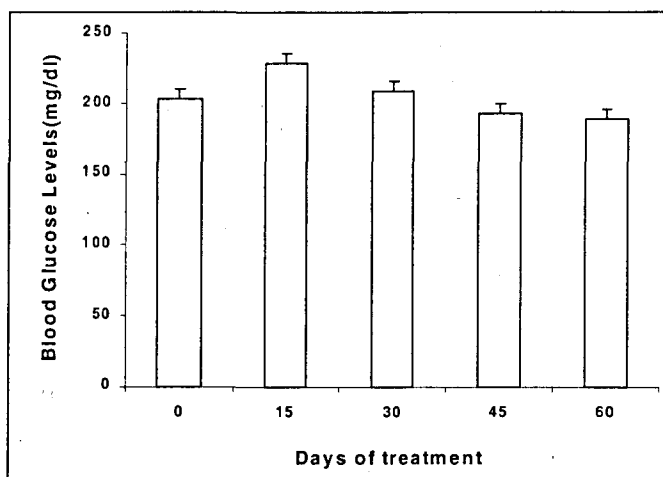

Fig. 1: Fasting blood glucose levels of diabetic patients before and after treatment with amalaki capsules.

The bars represent Mean±standard deviation of sample size of 10. The figure suggests that the decrease in blood glucose levels is statistically insignificant (p>0.05)
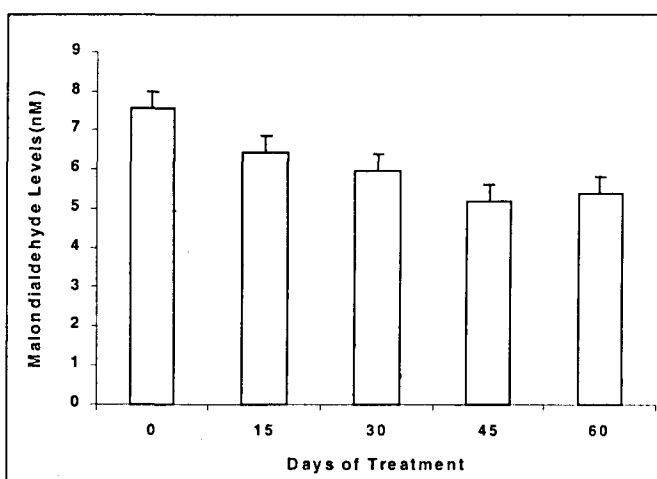

Fig. 2: Malondialdehyde levels of diabetic volunteers before and after treatment with amalaki capsules.

The bars represent Mean±standard deviation of sample size of 10. The figure suggests that the decrease in Malondialdehyde levels in diabetics is statistically significant (p< 0.05)

diabetic patients does not cause significant reduction of blood glucose levels. However, the drug can be beneficial to such patients since it decreases the levels of lipid peroxides significantly thus reducing the complications that may result from high levels of blood glucose. It has been proven that Diabetics have an increased need for the antioxidant vitamins like vitamin C and vitamin E. The drugs and diet rich in vitamin E and vitamin C improve the antioxidant status and also control blood sugar levels in diabetic patients[16]. However the present study has indicated that there is no direct relationship between blood glucose and lipid peroxide levels (malondialdehyde levels) in type 2 diabetic patients, but the *amalaki* capsules have shown significant antioxidant activity by reduction in lipid peroxide levels.

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