Effect of continuous administration of dried 75% methanolic extract of fruits of *Terminalia belerica* (Combretaceae) suspended in water was studied in alloxan induced hyperglycemia and antioxidant defense mechanism in rats. *T. belerica* prevented alloxan-induced hyperglycaemia significantly from 6th day of administration and there was 54% reduction on 12th day. Oxidative stress produced by alloxan was found to be significantly lowered by the administration of *T. belerica* extract. This was evident from a significant decrease in thiobarbituric acid reactive substances, conjugated dienes and hydroperoxides in blood and liver respectively. Similarly, decreased glutathione level produced by alloxan was increased by the administration of the extract in blood and liver. However the increase was not significant. Superoxide dismutase which was decreased by alloxan was significantly increased from 9th day in blood and liver of drug treated group. Similarly there was significant increase in the activity of catalase in blood and liver. Decrease in glutathione peroxidase by alloxan administration was found to be increased significantly in the blood and liver from 9th day by extract treatment. Glutathione reductase also was found to be increased in blood and liver. These results suggested that *T. belerica* fruit extract possessed anti-diabetic and anti-oxidant activity and these activities may be interrelated.

**Keywords:** Alloxan, Antidiabetic, Antioxidant enzymes, *Terminalia belerica*

Oxidative stress has significant effect in the causation of diabetes as well as diabetic related complications in human beings. In diabetes the oxidative stress co-exists with a reduction in the antioxidant status. The exact mode of action of oxidative stress in human diabetes is not known. Oxidative stress in diabetes increases glycation of proteins, inactivation of enzymes, alterations in structural functions of collagen basement etc. Oxidative stress has significant effect in the glucose transport protein (GLUT) and in insulin receptor activity. It is known that scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes and may alleviate the diabetes and reduces its secondary complications.

Several antioxidants of plant origin have been screened for their ability to scavenge free radicals and are useful as protective agents against oxidative stress. In a preliminary study it was observed that *Terminalia belerica* extract, which is used in the traditional medicine to reduce the serum glucose level, had significant antioxidant activity *in vitro* as it found to significantly reduce lipid peroxidation, scavenge hydroxyl radical and superoxide radicals *in vitro*. *T. belerica* has also been found to inhibit radiation induced lipid peroxidation. In fact, antioxidant activity of *T. belerica* was found to be superior to α-tocopherol. Alloxan, has been shown to produce diabetes by damaging of islet cells of pancreas by the liberated oxygen radicals. *Terminalia belerica* along with *Emblica oficinalis* and *Terminalia chebula* known as ‘Triphala’, is used for several diseases including diabetes. In the present study the anti-diabetic action of *T. belerica* and its relationship with its antioxidant activity is reported.

**Materials and Methods**

Preparation plant extract — The fruits of *Terminalia belerica*. Roxb were collected from Thrissur and identified against voucher specimen (No: Com 3) kept at Amala Ayurvedic Centre, Thrissur. Fruits were dried under 50°C, and the pericarp was powdered and extracted twice with 75% methanol overnight. This solvent system can extract the phenolic constituents in the seed which is mainly responsible for its antioxidant activity. The extract was evaporated to dryness under vacuum. Extract was resuspended in distilled water and used for the animal experiment. The yield of the extract was 9.8%.
Animals and chemicals — Male Wistar rats weighing 250-300g used in the experiment were housed in polypropylene cages at room temperature (25°-30°C) and had free access to drinking water and basal diet. The entire animal experiments were done as per the guidelines on Institutional Animal Ethics Committee. Alloxan was purchased from Sigma Chemicals (St Louis, Mo, USA). Nitroblue tetrizolium, oxidised glutathione and deoxy ribose were purchased from SRL Chemicals, Mumbai. All other chemicals used were of analytical reagent grade.

Experimental procedure — Rats (66) were divided into three groups. Group I, normal animals (6) were untreated. Group II, control animals (30) received freshly prepared alloxan in normal saline, ip as a single dose of 120 mg/kg body weight on day zero. They were further administrated with 1 ml of distilled water (vehicle) orally everyday during the experimental period. Group III, animals (30) were treated with alloxan as in group II. They were treated with 100 mg/kg body weight of T. bellerica extract, in 1ml water orally (once daily), starting from the same day of alloxan administration (day 1). As the control animals were also found to reduce their blood sugar, experiment was terminated on day 12.

Group I animals were sacrificed on the third day. Six animals each from group II and III, were sacrificed on 1, 3, 6, 9 and 12th day after drug treatment by pentothal sodium anesthesia. Blood was collected by heart puncture and serum and erythrocytes were separated. The liver was washed thoroughly and kept in -20°C till the analysis were completed.

Biochemical analysis — Serum glucose levels were estimated by GOD/POD enzymatic method of Trinder. A 10% w/v of liver homogenate was prepared in PBS (50 mM, pH 7) for the enzyme assay. Lipid peroxidation (LPO) level in liver was estimated using thiobarbituric acid (TBA) method of Ohkawa et al., by using 1,1,3,3 tetramethoxy propane as standard and the serum lipid peroxidation was estimated by the method of Satoh. Hydroperoxide was estimated by the method of Recknagel and Ghoshal. Conjugated diene was estimated by the method of Buege and Aust. Glutathione were estimated both in blood and liver tissue by the method of Moron et al. based on the reaction with DTNB and values were calculated from the standard of GSH treated with the same reagent.

Erythrocytes were prepared by the method of Minami and Yoshikawa and superoxide dismutase was estimated by the modified method of McCord and Fridovich. Catalase was estimated in the erythrocytes and liver tissue by the method of Aebi by measuring the rate of decomposition of hydrogen peroxide (H₂O₂) at 240 nm. A decrease in absorbency was observed after the addition of H₂O₂ to the reaction mixture containing either the tissue homogenate or the erythrocyte sediment which used as the source of catalase. Serum and tissue glutathione reductase was estimated by the method of Racker, which is based on the amount of reduced form nicotinamide adenosine dinucleotide phosphate (NADPH) consumed during the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH). The decrease in absorbence/min was noted and followed at every 1 min interval for 5 min at 340 nm and the concentration was calculated from the E₅₄₀ of nicotinamide adenosine dinucleotide phosphate (NADP). Glutathione peroxidase was estimated by the method of Paglia and Valentine based on the degradation of H₂O₂ in the presence of GSH. Protein content of the enzyme was determined by Lowry's method. Haemoglobin content of the blood was estimated by cyanmethemoglobin method using Drabkin's solution.

Statistical analysis — Statistical analysis was done using the Student’s ‘t’ test for glucose estimation and the in vivo antioxidant data were analyzed as per Bartett’s test of ANOVA using one way classification.

Results

Administration of T. bellerica extract did not have any significant effect on serum glucose level in alloxan diabetic rats during first five days. However as compared with untreated controls, animals treated with T. bellerica showed much lowered serum glucose level from day 6 onwards. On 9th day serum glucose in extract treated animals was found to be reduced to 54% (P<0.001) when compared with that of control diabetic animals (Fig. 1).

Serum lipid peroxidation level was found to be significantly elevated in diabetic animals when compared to normal (Table 1). Increased lipid peroxidation in diabetic animals was found to be reduced by administration of T. bellerica extract. Liver LPO was also found to be significantly increased in diabetic control group when compared with normal. Continuous administration of the T. bellerica extract was found to reduce the liver LPO levels significantly.
Hydroperoxides in the blood were found to be increased in untreated diabetic group from 3rd day onwards. Treatment with *T. belerica* extract significantly reduced the elevated level. Liver hydroperoxides was also found to be elevated in diabetic animals significantly from 3rd day. Treatment with *T. belerica* reduced the hydroperoxide level significantly and became almost normal on 12th day. Conjugated diene was significantly increased both in blood and liver tissue in alloxan diabetic animals. These elevated levels were reduced significantly both in blood and liver from day 6 in the *T. belerica* treated animals (Table 1). These results indicate that *T. belerica* extract could significantly inhibit the oxidative stress induced in the animals produced by alloxan administration.

GSH level in erythrocytes as well as in the liver did not produce any significant change in any of the groups of animals when compared to normal animals (Table 2). The superoxide dismutase activity was found to be reduced significantly in erythrocytes of animals treated

| Table 1—Effect of *T. belerica* on lipid peroxidation (LPO), hydroperoxide (HP) and conjugated diene (CD) in blood and liver tissue in alloxan induced diabetic rats |
|-----------------------------|--------------------|-----------------------------|
|                             | Normal             | Alloxan                    |
| LPO (nmol/ml)               | 1.27±0.13<sup>b</sup> | 1.41±0.14<sup>*</sup>       | 1.32±0.06<sup>*</sup> |
| Serum (U/g)                 | 1.65±0.22<sup>**</sup> | 1.93±0.42<sup>**</sup>     | 1.29±0.13<sup>**</sup> |
| Liver (nmol/mg protein)     | 0.862±0.03<sup>**</sup> | 0.898±0.02<sup>**</sup>   | 0.845±0.02<sup>**</sup> |
| HP (mm/100g tissue)         | 10.01±0.90<sup>b</sup> | 19.02±3.23<sup>**</sup>   | 12.65±1.11<sup>**</sup> |
| Liver (nmol/mg protein)     | 1.81±0.21<sup>**</sup> | 2.46±0.18<sup>**</sup>    | 1.03±0.07<sup>**</sup> |
| CD (mm/100g tissue)         | 26.57±4.69<sup>**</sup> | 26.57±4.69<sup>**</sup>   | 26.57±4.69<sup>**</sup> |
| CD (U/g Hb)                 | 1.02±0.07<sup>**</sup> | 1.71±0.24<sup>**</sup>    | 1.30±0.07<sup>**</sup> |
| Liver (mm/100g tissue)      | 25.36±5.72<sup>**</sup> | 25.36±5.72<sup>**</sup>   | 25.36±5.72<sup>**</sup> |

A=day 3; B=day 6; C=day 9 ; D=day 12.
Alphabets (a,b,c) indicate the result of ANOVA test comparing between the groups. Different alphabets are independently significant. *P*: <0.001; ** <0.001
with alloxan from 9\textsuperscript{th} day. Administration of \textit{T. belerica} regained SOD activity. Similarly, in liver the SOD was found to be significantly decreased in diabetic control group from 9\textsuperscript{th} day onwards. In \textit{T. belerica} treated group, there was significant increase in the SOD activity (Table 2).

Catalase activity in erythrocytes from diabetic rats was found to be significantly decreased from 6\textsuperscript{th} day as compared to normal. It was found that in \textit{T. belerica} treated animals catalase activity was found to be significantly increased. Liver catalase which was lower in diabetic group was found to be significantly elevated in \textit{T. belerica} treated group (Table 2).

The blood glutathione peroxidase activity was found to be significantly lowered from 3\textsuperscript{rd} day onwards in untreated diabetic group while it was found to be significantly higher after administration of \textit{T. belerica} (Table 3). Similarly liver GPx was found to lowered in alloxan diabetic group whereas the levels were significantly higher in extract treated group. Serum glutathione reductase showed significant reduction from 9\textsuperscript{th} day when compared to normal. Administration of \textit{T. belerica} increased the level significantly from day 9. Liver GR was found to be significantly reduced from 3\textsuperscript{rd} day in diabetic animals and continuous administration of the \textit{T. belerica} extract elevated this level (Table 3). In summary, alloxan induced significant oxidative stress was reduced by the treatment of \textit{T. belerica}.

**Discussion**

\textit{Terminalia belerica} extract (100 mg/kg, body weight) significantly decreased serum glucose level in hyperglycaemic animals. Alloxan administration produced elevated level of lipid peroxidation, hydroperoxides and conjugated diene which is a clear manifestation of excessive formation of free radicals.
resulting in tissue damage. Karpen et al. observed an elevated level of lipid peroxides in the plasma of streptozotocin diabetic rats. Significant decline in the concentration of these constituents in the liver tissue and serum of *T. belerica* treated diabetic animals indicate the potential use of the extract *in vivo* to counteract the oxidative stress induced changes in diabetes.

Hyperglycemia, defining established diabetes, can induce oxidative stress by various mechanisms. Excessive levels of glucose reaching mitochondria lead to an overdrive of the electron transport chain, resulting in overproduction of superoxide anions normally scavenged by mitochondrial SOD. When the latter fails, oxidative stress develops and it was proposed that this mechanism is responsible for the activation of all major diabetic complications involving glycation, sorbitol pathway etc. The *in vitro* supplementation of SOD like drugs corrected most of these defects, supporting importance of these mechanisms.

Oxidative stress acts as secondary messenger and through signal transduction via NF-κB, affects gene expression, thereby the expression of antioxidant enzyme may be reduced. Moreover hyperglycemia can simply inactivate existing enzymes by glyating these proteins, e.g., glycation of SOD, CAT. Decrease in CAT, SOD and GPx activity was also observed in diabetic animals which were increased as an effect of *T. belerica* extract supplementation.

GSH is a major non-protein thiol in living organisms which plays a central role in co-ordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In the present study, decline in the activities of these enzymes in alloxan-induced animals and attainment of normalcy in *T. belerica* treated rats indicate that oxidative stress elicited by alloxan was significantly reduced by this extract.

Many natural antioxidants such as ellagic acid and gallic acid are reported to present in the fruits of *T. belerica*. Some major triterpinoids---arjungenin, bellericagenin and belleric acid were also isolated from the plant materials. However, their pharmacological activity has not been reported. Natural antioxidants strengthen the endogenous antioxidant defenses against reactive oxygen species (ROS) and restore the optimal balance. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *T. belerica* can rightly be mentioned as a plant of considerable importance.

**Acknowledgement**

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