Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Emblica officinalis improves glycemic status and oxidative stress in STZ induced type 2 diabetic model rats

Aneesa Ansari[1], Md. Shahed Zaman Shahriar[2], Md. Mehedi Hassan[2,5], Shukla Rani Das[3], Begum Rokeya[4], Md. Anwarul Haque[2], Md. Enamul Haque[6] , Nirupam Biswas[7*], Tama Sarkar[8]

[1]*Department of Genetic Engineering and Biotechnology, Jessore Science and Technology University, Jessore, Bangladesh*
[2]*Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh*
[3]*Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh*
[4]*Department of Biochemistry and Cell Biology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh*
[5]*Department of Biomedical Science, Chonbuk National University Medical School, Jeonju 561-712, South Korea*
[6]*Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000,Bangladesh*
[7]*Department of Nutrition and Food Sciences, University of Huddersfield, Huddersfiled, HD1 3DH, United Kingdom*
[8]*Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh*

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 15 October 2013
Accepted 15 December 2013
Available online 20 January 2014

Keywords:
Antidiabetic
Antioxidant
Emblica officinalis
Non-insulin dependent diabetes mellitus
Type 2 diabetic rats
Reduced glutathione

ABSTRACT

Objective: To evaluate the antidiabetic and antioxidant potential of *Emblica officinalis* (*E. officinalis*) fruit on normal and type 2 diabetic rats. **Methods:** Type 2 diabetes was induced into the male Long-Evans rats. The rats were divided into nine groups including control groups receiving water, type 2 diabetic controls, type 2 diabetic rats treated with glibenclamide (T2GT) and type 2 diabetic rats treated with aqueous extract of fruit pulp of *E. officinalis*. They were fed orally for 8 weeks with a single feeding. Blood was collected by cutting the tail tip on 0 and 28 days and by decapitation on 56 day. Packed red blood cells and serum were used for evaluating different biochemical parameters. **Results:** Four weeks administration of aqueous extract of *E. officinalis* improved oral glucose tolerance in type 2 rats and after 8 weeks it caused significant ($P<0.007$) reduction in fasting serum glucose level compared to 0 day. Triglycerides decreased by 14% but there was no significant change in serum ALT, creatinine, cholesterol and insulin level in any group. Furthermore, reduced erythrocyte malondialdehyde level showed no significant change ($P<0.07$) but reduced glutathione content was found to be increased significantly ($P<0.05$). **Conclusions:** The aqueous extract of *E. officinalis* has a promising antidiabetic and antioxidant properties and may be considered for further clinical studies in drug development.

1. Introduction

Diabetes mellitus type 2 is a metabolic disorder, and has become a thoughtful problem of modern society due to the severe long term health complications associated with it. This is the most encountered form of diabetes, accounting for more than 80% of the total cases of diabetes[1]. Oxidative stress in diabetes coexists with a reduction in the antioxidant status[2], which ultimately increases the deleterious effects of free radicals[3]. Streptozotocin (STZ) induces its diabetogenic activity mainly by inducing oxygen free radical and causing necrosis of the pancreas[3]. Both the radical and non-radical oxidants can induce lipid peroxidation particularly of those lipoproteins that contain unsaturated fatty acids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen, basement and other membranes and play a role in the long term complications of diabetes[4,5]. Increased oxidative stress in diabetes mellitus may also be a reason for such decrease in erythrocytes count. Hyperglycemia can burden the cells with extra free radicals[6]. This coupled with reduced glutathione (GSH) content secondary to its increased utilization in diabetic erythrocytes can cause peroxidative

*Corresponding author: Nirupam Biswas, Department of Nutrition and Food Sciences, University of Huddersfield, Huddersfiled, HD1 3DH, United Kingdom.
Tel: +447745738807.
E-mail: nirupamgene@gmail.com

breakdown of phospholipids fatty acids in the erythrocytes membrane[7]. It is noted that erythrocytes of diabetic patients are more susceptible to lipid peroxidation when treated with hydrogen peroxide *in vitro*[8].

Antioxidants play an important protective role against the reactive oxygen species. Reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[9].

Emblica officinais (*E. officinalis*; Family: Euphorbiaceae) are used in Ayurveda as a potent rasayanas, a class of plant-derived drugs reputed to promote health and longevity by increasing defense against diseases[10,11]. *E. officinalis* has been reported as a rich source of vitamin C, which plays an important role in scavenging free radicals[3]. This study was designed to evaluate the antidiabetic and antioxidant activities of *E. officinalis* in streptozotocin induced type 2 diabetic model rats.

2. Materials and methods

2.1. Materials and aqueous extract of E. officinalis

E. officinalis was purchased from the local market. The pulp of the fruit was separated from the seed and blended. The resultant juice was filtered with a clean cloth and the supernatant separated. The aqueous extract was evaporated to concentrate at reduced pressure using a rotary vacuum evaporator at 50 ℃ and finally dried using freeze-drier.

2.2. Rats and diets

The experimental protocol was approved by the Animal Studies committee of BIRDEM. Adult male Long Evans rats weighing approximately 180-220 g were obtained from BIRDEM Animal House, Dhaka, Bangladesh. The 32 rates were randomly divided into four groups: normal control group (n=8) fed with deionized water at a dose of 10 mL/kg body weight; non-insulin dependent diabetes mellitus (NIDDM) control group (n=8) fed with deionized water at a dose of 10 mL/kg body weight; NIDDM positive control group(n=8) treated with glibenclamide at a dose of 5 mg/10 mL (9.9 mL H_2O + 0.1 mL Twin 20)/kg body weight; NIDDM treated group (n=8) fed with aqueous extract of *E. officinalis* at a dose of 1.25 g/10 mL/ kg body weight. The animals were bred at BIRDEM Animal house, Dhaka, Bangladesh maintained at a constant room temperature of (22±5) ℃ with humidity of 40%-70% and the natural 12 h day-night cycle. The rats were fed on a standard laboratory pellet diet and water supplied ad libitum.

Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 90 mg/kg body weight) in citrate buffer (10 mL) to the 48 h old rat pups (approximately 7 g) as described by Bonner-Weir *et al*[12]. Experiments were carried out 3 months after STZ injection and rats having blood glucose level 8-12 mmol/L at fasting conditions, these were taken to carry out the experiments. Body weight, food and water intakes were observed at regular intervals for all rats.

2.3. Biochemical analysis

Blood samples were collected on 0 day from the tail tips for different biochemical analysis, on the 28th day post-prandial blood glucose level was determined and the rats were decapitated on the 56 day after 12 h fasting. Blood samples were collected in heparinized tubes for estimation of malondialdehyde (MDA) and reduced GSH. Serum glucose was estimated by glucose oxidase (GOD-PAP, Boheringer Mannheim GmbH), serum triglyceride and cholesterol by enzymatic-colorimetric, creatinine and alanine aminotransferase (ALT) by Automatic Analyzer and serum insulin by an ELISA. Packed red cells were used for the estimation of MDA by using the thiobarbituric acid method and GSH was assayed by Ellman's method[13].

2.4. Statistical analysis

All data sets were expressed as mean±SD or as median (Range) as appropriate. Data were also statistically analyzed using the student's t-test (paired and unpaired) or ANOVA (analysis of variance) followed by Bonferroni *post hoc* test or Mann Whitney (U) test. The differences were considered significant at $P<0.05$.

3. Results

3.1. Effect of aqueous extract of E. officinalis on glucose homeostasis

Results of fasting serum glucose (FSG) level of the studied rats at baseline (before onset of feeding *i.e.*, 0 day) and 56 day of feeding is presented in Table 1.

At baseline FSG (mmol/L) of type 2 water, glibenclamide and aqueous extract of *E. officinalis* treated groups were almost similar, whereas in normal control rats FSG was (7.96 ±0.27) mmol/L. As it is seen from the Table 1, on 56 day the FSG level of all the groups of rats decreased, although the decrease was not significant in normal rats. Type 2 rats fed with aqueous extract of *E. officinalis* showed a significant decrease while comparing within groups (t=6.497, P=0.007). As expected, glibenclamide also ameliorated the diabetic condition on 56 day (t=4.148, P=0.025). Type 2 control rats also significantly reduced blood glucose level (t=5.740, P=0.01). While comparing within groups, only glibenclamide

showed a significant reduction in serum glucose level on 56 day in comparison with normal rats.

Table 1
Chronic effect of aqueous extract of *E. officinalis* on fasting blood glucose levels of normal and type 2 diabetic model rats.

Group	Glucose (mmol/L)	
	0 day	56 day
NWC	7.96±0.27	6.60±0.91
Type 2 WC	10.01±1.27	6.29±1.19
Type 2 GT	9.91±2.24	5.27±0.47
Type 2 AE	10.23±1.36	6.75±0.50

Data were expressed as Mean±SD. NWC: Normal water control, NAE: Normal aqueous extract of *E. officinalis*, Type 2 WC: Type 2 water control, Type 2 GT: Type 2 glibenclamide positive control, Type 2 AE: Aqueous extract of *E. officinalis* type 2 group.

3.2. Effect of aqueous extract of E. officinalis on the postprandial blood glucose level of type 2 diabetic model rats when fed simultaneously with glucose load performed on 28 day of study period

Table 2 shows the acute effect of aqueous extract of *E. officinalis* on the postprandial blood glucose level on different groups of type 2 diabetic model rats when extract was fed simultaneously with the glucose load on 28 day. As it was seen fasting serum glucose level of the type 2 extract fed groupwas higher [(9.38±1.10) mmol/L], while the rest of the groups showed almost same level of blood glucose. As it is seen, the percentage increase of serum glucose was 62%, 122%, 148% and 64% in normal rats, type 2 water control rats, glibenclamide treated and extract treated groups, respectively (at 30 minutes) and at 90 minutes the rise was 26%, 122%, 158% and 70%, respectively. Although the fasting glucose level of the extract treated groups was significantly higher than the rest of the groups on day 28, it opposed the rise of blood glucose at both the time points.

Table 2
Effect of aqueous extract of *E. officinalis* on the postprandial blood glucose level of type 2 diabetic model rats when fed simultaneously with glucose load performed on the 28th day of study period.

Group	Glucose (mmol/L)		
	0 min	30 min	90 min
NWC	6.76±0.64	10.96±3.31	8.51±1.04
Type 2 WC	7.43±0.82	16.50±1.70	16.48±3.91
Type 2 GT	7.01±2.23	17.38±2.62	18.10±2.61
Type 2 AE	9.26±0.95	15.22±3.58	15.75±1.01

Data were expressed as Mean±SD. NWC: Normal water control, NAE: Normal aqueous extract of *E. officinalis*, Type 2 WC: Type 2 water control, Type 2 GT: Type 2 glibenclamide positive control, Type 2 AE: Aqueous extract of *E. officinalis* type 2 group.

3.3. Chronic effect of aqueous extracts of E. officinalis on the serum cholesterol and triglyceride levels of normal and type 2 diabetic model rats

As shown in Table 3, the effect of aqueous extract of *E. officinalis* on the total serum cholesterol and triglyceride levels of normal water treated and type 2 diabetic model rats. It is evident that type 2 water treated group had an increase of 8% in the total serum cholesterol level at the end of study period. However, glibenclamide treated type 2 rats showed a decrease in the total serum cholesterol after 56 days feeding by 4%. In case of the type 2 treated extract group, there was a 5% increase in comparison to the 0 day value. Virtually, there was no significant change in the total cholesterol level at the end of the study period.

In case of serum triglyceride level, it is seen that other than type 2 water treated group (which has in increase in of 1%), the rest had a fall in the triglyceride levels (*i.e.* 20% decrease in normal water fed rats, 8% in type 2 glibenclamide treated rats and 14% in extract treated type 2 rats).

Table 3
Chronic effect of aqueous extracts of *E. officinalis* on the serum cholesterol and triglyceride levels of normal and type 2 diabetic model rats.

Group	Cholesterol (mg/dL)		TG (mg/dL)	
	0 day	56 day	0 day	56 day
NWC	61±5	62±8	69±6	55±19
Type 2 WC	60±4	65±8	56±15	57±10
Type 2 GT	60±7	58±10	66±19	61±19
Type 2 AE	56±5	59±5	64±19	55±3

Data were expressed as Mean±SD. NWC: Normal water control, NAE: Normal aqueous extract of *E. officinalis*, Type 2 WC: Type 2 water control, Type 2 GT: Type 2 glibenclamide positive control, Type 2 AE: Aqueous extract of *E. officinalis* type 2 group.

3.4. Chronic effect of aqueous extracts of E. officinalis on creatinine and ALT levels of normal and type 2 diabetic model rats

Effect on serum creatinine and ALT is presented in Table 4. Serum creatinine and ALT levels remained steady in all the groups. No significant change was noticed in serum creatinine and ALT levels after 56 day of the study period.

Table 4
Chronic effect of aqueous extracts of *E. officinalis* on creatinine and alt levels of normal and type 2 diabetic model rats.

Group	Creatinine (mg/dL)		ALT (mg/dL)	
	0 day	56 day	0 day	56 day
NWC	0.70±0.04	0.90±0.04	72±24	53±7
Type 2 WC	0.80±0.00	0.90±0.01	52±7	53±9
Type 2 GT	0.80±0.05	0.80±0.07	55±14	58±15
Type 2 AE	0.80±0.04	0.90±0.02	62±11	51±4

Data were expressed as Mean±SD. NWC: Normal water control, NAE: Normal aqueous extract of *E. officinalis*, Type 2 WC: Type 2 water control, Type 2 GT: Type 2 glibenclamide positive control, Type 2 AE: Aqueous extract of *E. officinalis* type 2 group.

3.5. Chronic effect of aqueous extract of E. officinalis on the serum Insulin level of normal and type 2 diabetic model rats

Chronic effect of aqueous extract of *E. officinalis* on the

insulin level of normal rats fed with water and different groups of type 2 diabetic model rats is shown in Table 5. It is clearly seen that all the groups of type 2 rats had a significantly lower insulin level on 0 day, in comparison to normal rats. On day 56, serum insulin increased in normal control groups by 14%. Although reduction in serum insulin level was noticed in different groups of type 2 rats at the end of study period, the reduction was highest in type 2 control group (53% reduction) in glibenclamide treated group showed a fall of 15% while *E. officinalis* treated Type 2 rats had a reduction of 21%.

Table 5

Chronic effect of aqueous extract of *E. officinalis* on the serum insulin level of normal and type 2 diabetic model rats.

Group	Insulin (ng/dL)	
	0 day	56 day
NWC (n=6)	1.32±0.34	1.51±1.26
Type 2 WC (n=5)	0.51±0.21	0.24±0.13
Type 2 GT (n=5)	0.25±0.04	0.21±0.06
Type 2 AE (n=6)	0.48±0.26	0.38±0.17

Data were expressed as Mean±SD. NWC: Normal water control, NAE: Normal aqueous extract of *E. officinalis*, Type 2 WC: Type 2 water control, Type 2 GT: Type 2 glibenclamide positive control, Type 2 AE: Aqueous extract of *E. officinalis* type 2 group.

3.6. Chronic effect of aqueous extract of E. officinalis on erythrocyte MDA and reduced GSH levels of experimental rats

The table 6 shows that the concentration of erythrocyte lipid peroxidation products *i.e.* MDA and reduced GSH in different groups of rats after 56 days of the study period. As expected, type 2 diabetic control rats exhibited the highest level of lipid peroxidation: erythrocyte MDA level was 27.9 (median, nmol/g Hb) whereas normal rats had 19.9 nmol/g Hb. The levels of erythrocyte MDA was significantly lower compared to type 2 glibenclamide treated group (P=0.01). *E. officinalis* extract administration to type 2 rats also tend to normalize the erythrocyte MDA level (median=20.4 nmol/g Hb) but the change remained just outside the significant level (P=0.078).

As it is seen from the table, the levels of the major cellular antioxidant GSH was lower in type 2 control rats (median=5.31 mg/g Hb). The decrease GSH content contributes to the pathogenesis of complications associated with chronic diabetic state. GSH level increased significantly in *E. officinalis* extract administrated type 2 rats (median=5.9 mg/g Hb, P=0.055). Glibenclamide treated rats also had a higher GSH level.

Table 6

Effect of aqueous extracts of *E. officinalis* on erythrocyte MDA and reduced GSH levels of normal and type 2 diabetic model rats treated for 2 months.

Group	MDA (nmol/g Hb)	GSH (mg/gm Hb)
NWC (n=6)	19.90±0.17	5.40±0.33
Type 2 WC (n=6)	27.90±0.23*	5.30±0.51
Type 2 GT (n=8)	18.20±0.42$^{\triangle}$	5.60±0.07
Type 2 AE (n=6)	20.40±0.13	5.90±0.29

Data were expressed as Mean±SD. NWC: Normal water control, NAE: Normal aqueous extract of *E. officinalis*, Type 2 WC: Type 2 water control, Type 2 GT: Type 2 glibenclamide positive control, Type 2 AE: Aqueous extract of *E. officinalis* type 2 group.

*: P<0.05, comparing with NWC; $^{\triangle}P$<0.05, comparing with Type 2 WC.

4. Discussion

Diabetes produced by administration of STZ (a β-cell toxic agent) usually causes destruction of pancreatic β-cells[3]. This later improves degranulation and reduction of insulin secretion accompanying with hyperglycemia. The present study demonstrated that administration of *E. officinalis* for 8 weeks resulted in the significant (P<0.007) reduction of fasting serum glucose level compared to baseline level. The study also revealed that the reduction in serum glucose level was gradual. After 4 weeks administration of aqueous extract of Amla extract significantly increased postprandial serum glucose level whereas the fasting serum glucose level remain unchanged in type 2 diabetes rats. Postprandial serum glucose increased by 62% in normal control, 122% in diabetic control and 148% in glibenclamide treated and 64% in *E. officinalis* treated diabetic rats. Hence, the finding clearly demonstrates that aqueous extract of *E. officinalis* improves oral glucose tolerance in type 2 rats[10,14]. It is now well established that dyslipidemia plays an important role in the development of diabetic complication. The effects on diabetic complication were assessed by measuring the atherogenic lipids (*i.e.* total cholesterol and triglycerides) after chronic feeding of *E. officinalis* to diabetic rats. The obtained results demonstrated that total cholesterol level did not change by *E. officinalis* treatment. Conversely, Triglycerides level decreased 14% by administration of *E. officinalis* for 8 weeks[15,16].

This study was carried out on STZ induced type 2 model rats which were hypoinsulinemic. Insulin level of normal rats were (1.32±0.34) ng/dL, whereas all other groups had almost 3 fold less insulin level. Eight weeks treatment with *E. officinalis* did not improve serum insulin level in type 2 diabetic rats. The only difference in *E. officinalis* treated animals was less marked in insulin content (0 day: 0.48±0.26 versus 56 day: 0.38±0.17) comparison to type 2 control rats [0 day: (0.51±0.21) ng/mL Versus 56 day: (0.24±0.13) ng/mL). Therefore, it is revealed that slowly generated hypoglycemic effect of *E. officinalis* STZ-induced type 2 rats may involve an extra pancreatic effect since serum insulin level did not increase. *E. officinalis* was used at a dose of 1.25 g/b.w. To

exclude any toxic effect of aqueous extract of Amla was measured on liver and kidney function, serum ALT and serum creatinine level were measured at the beginning and at the end of the study period. No significant change was noticed in serum ALT and creatinine level in any group of study rats after 8 weeks study period. Therefore, the results is exposed that eight weeks administration of *E. officinalis* had no toxic effect[14].

Hypoinsulemia in diabetes increases the activity of acyl co-enzyme A oxidase which initiates β-oxidation of fatty acids resulting in lipid peroxidation[17] which is determined by rhiobarbuturic acid reactive (TBAR) substances level. Baynes (1991) and Kakkar *et al* (1995) reported that tissue blood malondialdehyde levels of rats with STZ induced diabetes increased due to lipid peroxidation[5,18]. Jain *et al* (1999) reported that there is a significant increased membrane lipid peroxidation in diabetic erythrocytes compared with normal erythrocytes[19]. On the basis of these results, the present study evaluated MDA (TBAR) levels in erythrocyte homolysate to determine the degree of oxidative damage in diabetic rats and found that it was significantly higher in the diabetic control group compared with normal group (P=0.025). This result suggest that the increased malondialdehyde levels in diabetic control rats result in increased levels of reactive oxygen species, which attack poly unsaturated fatty acids in cell membranes, cause lipid peroxidation and subsequent development of diabetic complications. In contrast, orally administrated aqueous extract of *E. officinalis* reduced erythrocyte malondialdehyde levels in type 2 rats indicating that Amla could be a potent inhibitor of oxidative damage in erythrocytes[2,15].

In this study we evaluated the antidiabetic and antioxidant potential of *E. officinalis* fruit on type 2 diabetic rats. The aqueous extract of *E. officinalis* showed potential antidiabetic and antioxidant properties during treatment of type 2 diabetic rats. This might be considered for further clinical studies in drug development.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to Dr. Begum Rokeya, Department of Pharmacology, BIRDEM (Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders), Shahbag, Dhaka, Bangladesh, for providing technical, moral and financial support for this project.

References

[1] Mlinar B, Marc J, Janez A. Pfeifer M. Molecular mechanisms of insulin resistance and associated diseases. *Clin Chim Acta* 2007; **375**: 20-35.

[2] Chandra RH, Veeresham C, Asres K. Effects of *Emblica officinalis* commercial formulation in type 2 diabetic patients. *Indian J Pharm Sci* 2004; **66**: 735-738.

[3] Nain P, Saini V, Sharma S, Nain J. Antidiabetic and antioxidant potential of *Emblica officinalis* Gaertn. leaves extract in streptozotocin-induced type-2 diabetes mellitus (T2DM) rats. *J Ethnopharmacol* 2012; **142**: 65-71.

[4] Violi F, Marino R, Milite MT, Lofrredo L. Nitric oxide and its role in lipid peroxidation. *Diabetes Metab Res Rev* 1999; **15**: 283-288.

[5] Baynes, JW. Role of oxidative stress in diabetes of complication, India. *Diabetes* 1991; **40**: 405-412.

[6] Jain SK, McVie R, Jaramillo JJ, Chen Y. Hyperketonemia (acetoacetate) increases the oxidizability of LDL + VLDL in type 1 diabetic patients. *Free Radical Bio Med* 1998; **24**: 175-181.

[7] Jain SK, McVie R. Effect of glycemic control race (white vs black), and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients. *Metabolism* 1994; **43**: 306-309.

[8] Uzel N, Sivas A, Uysal M, Oz H. Erythrocyte lipid peroxidation and glutathione peroxidase activities in patients with diabetes mellitus. *Horm Metab Res* 1987; **19**: 89-90.

[9] Sies H. Strategies of antioxidant defense. *Eur J Biochem* 1993; **215**: 213-219.

[10] Akhtar MS, Ramzan A, Ali A, Ahmad M. Effect of Amla fruit (*Emblica officinalis* Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. *Int J Food Sci Nutr* 2011; **62**: 609-616.

[11] Udupa, KN, Singh RH. Clinical and experimental studies on Rasayana drugs and Panchkarma therapy, Central Council for Research in Ayurvada and Siddha, New Delhi; 1995.

[12] Bonner-Weir S, Trent DF, Honey RN, Weir GC. Responses of neonatal rat islets on streptozotocin limited beta cell regeneration and hyperglycemia. *Diabetes* 1981; **30**: 64-69.

[13] Ellman GL. Tissue sulfhydril groups. *Arch Biochem Biophys* 1959; **82**: 70-77.

[14] Rao TP, Sakaguchi N, Juneja LR, Wada E, Yokozawa T. Amla (*Emblica officinalis* Gaertn.) extracts reduce oxidative stress in streptozotocin-induced diabetic rats. *J Med Food* 2005; **8**: 362-368.

[15] Shikha M, Singh RK, Jaiswal D, Rai PK, Watal G. Anti-diabetic activity of *Emblica officinalis* in animal models. *Pharmaceutical Biol* 2009; **47**: 1050-1055.

[16] Shamim A, Qureshi, Asad W, Sultana V. The effect of *Phyllantus emblica* Linn on type-[] Diabetes, triglycerides and liver -specific enzyme. *Pakistan J Nut* 2009; **8**: 125-128.

[17] Robinson AC, Burke J, Robinson S, Johnston DG, Elkeles RS. The effects of metformin on glycemic control and serum lipids in insulin-treated NIDDM patients with suboptimal metabolic control. *Diabetes Care* 1998; **21**: 701-705.

[18] Kakkar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. *Mol Cell Biochem* 1995; **18**: 113-119.

[19] Jain SK, McVie R. Hyperketonemia can increase lipid peroxidation and lower glutathione levels in human erythrocytes in vitro and in type 1 diabetic patients. *Diabetes* 1999; **48**: 1850-1855.