

BENEFICIAL EFFECTS OF TYROSOL ON PLASMA LIPID PEROXIDATION AND NON-ENZYMATIC ANTIOXIDANTS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Leelavinothan Pari* and Ramasamy Chandramohan

Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India.

Abstract

In the present research, we evaluated the beneficial effects of tyrosol on altered plasma lipid peroxides and non-enzymatic antioxidants in streptozotocin induced diabetic rats. Diabetes mellitus was induced in rats by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight). Streptozotocin induced diabetic rats were treated with tyrosol (5 mg, 10 mg and 20 mg/kg body weight) and glibenclamide (600 µg/kg body weight) orally once a day for a period of 45 days. Diabetic rats also showed a significant increase in plasma glucose and lipid peroxidation products and a significant decrease in plasma insulin and non-enzymatic antioxidants. Tyrosol treatment near normalized all the biochemical parameters studied. Tyrosol (20 mg/kg body weight) revealed the most significant effect than the other two doses. Thus, the present study exhibits the antilipid peroxidation and antioxidant effects of tyrosol in the circulation of streptozotocin induced diabetic rats.

Key words: Diabetes Mellitus, Streptozotocin, Tyrosol, Vitamin C, Glucose and Insulin.

1. Introduction

Diabetes mellitus (DM) is a leading cause of morbidity and mortality in the world's growing population. The prevalence of DM in adults worldwide is estimated to rise from 382 million in the year 2013 to 592 million in the year 2035 (IDF, 2013). The major part of this increase is expected to occur in developing countries, with the greatest absolute increase expected to be seen in India. According to the International Diabetes Federation, 65.1 million people in India had DM in 2013 (IDF, 2013). DM characterized by increased blood glucose level as a result of disrupted insulin-signaling, which leads to insulin deficiency resulting from autoimmune destruction of insulin-producing β -cells in the case of type-

1DM, or insulin resistance and declining β cell function in type-2 DM. Type-2 DM accounts for more than 90-95% of all cases of DM globally.

Persistent hyperglycemia in DM leads to an increase in the oxidative stress because of the overproduction of free radicals, especially reactive oxygen species (ROS). When the generation of ROS exceeds cellular defense mechanisms, the ROS interact with lipids, carbohydrates, proteins, DNA and membrane lipid peroxidation, which could cause structural changes as well as functional abnormalities (Selvaraj *et al.*, 2005). Increased oxidative stress is a well-known pathogenic mechanism related to the development and progression of DM and its complications (Rudge, 2007). In view of the above facts, therapeutic agents which can control hyperglycaemia as well as protection from oxidative stress are very much essential

*Corresponding author: **Leelevinothan Pari**

E-mail: jayampari@gmail.com

Received: 10.04.2015; Revised: 16.05.2015;

Accepted: 22.06.2015.



to lessen the diabetes complications (Calcutt, 2009). The streptozotocin (STZ) rat model of DM is one of the most commonly employed models of human disease (Ugarte, 2012), because it mimics many of the complications of human DM. It has been extensively used to induce diabetes mellitus in experimental rat models. The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage (Shrilatha and Muralidhara, 2007).

DM is often controlled by synthetic agents such as biguanides, sulfonylureas and α -glucosidase inhibitors, but they have several adverse effects (Posuwan, 2013). Hence, considerable attention has been focused on the use of dietary constituents and natural products as an alternative or complimentary treatment for diabetic medication to reduce the adverse effects caused by the synthetic medicines. Tyrosol (4-(2-hydroxyethyl) phenol) is a well-known phenolic compound. It is mainly present in extra-virgin olive oil and white wine (St-Laurent-Thibault, 2011). Previous studies have revealed the anti-cancer, anti-depressant, anti-inflammatory, cardioprotective, and neuro protective effects of tyrosol [Ahn *et al.*, 2008; Panossian *et al.*, 2008; Giovannini *et al.*, 1999; Chernyshov *et al.*, 2007; Bu *et al.*, 2007]. Since, altered lipid peroxidation and non-enzymatic antioxidants plays a vital role in the pathogenesis and complications of DM, we investigated the effect of tyrosol on altered plasma glucose, plasma insulin, plasma lipid peroxidation products and non-enzymatic antioxidants in STZ induced diabetic rats. The effects exerted by tyrosol are compared with that of glibenclamide.

2. Materials and Methods

Chemicals

Streptozotocin and tyrosol were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade and purchased from Hi Media and SD-Fine Chemicals, Mumbai, India.

Experimental Animals

Male albino Wistar rats, weighing about 180 - 220 g were procured from Central Animal House,

Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. They were housed in clean, sterile, polypropylene cages under standard vivarium conditions (12 h light/dark cycles) with free access to standard chow (Hindustan Lever Ltd., Bangalore, India) and water. The experimental protocol was approved by the Institutional Animal Ethical Committee, Annamalai University (Reg No. 1002, 2013).

Induction of Experimental DM

DM was induced in overnight fasted rats by a single intraperitoneal injection of STZ (40 mg/kg body weight) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). STZ injected rats were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. The induction of DM in rats was confirmed by estimating the elevated plasma glucose levels, 72 h after STZ injection. Rats with fasting plasma glucose levels more than 250 mg/dL were considered diabetic and chosen for the current study.

Experimental Design

A total of 42 rats (30 STZ-induced diabetic rats and 12 normal rats) were used and they were divided into 5 groups with 6 rats in each group as follows:

- Group I : Normal control rats.
- Group II : Normal rats given intra gastrically 1 mL of tyrosol (20 mg/kg body weight) dissolved in distilled water daily for 45 days.
- Group III : STZ-induced diabetic control rats.
- Group IV : STZ-induced diabetic rats treated with 1 mL of tyrosol (5 mg/kg body weight) intra gastrically dissolved in distilled water daily for 45 days.
- Group V : STZ-induced diabetic rats treated with



V 1 mL of tyrosol (10 mg/kg body weight) intra gastrically dissolved in distilled water daily for 45 days.

Group : STZ-induced diabetic rats treated with
VI 1 mL of tyrosol (20 mg/kg body weight) intra gastrically dissolved in distilled water daily for 45 days.

Group : STZ-induced diabetic rats treated with
VII 1 mL of glibenclamide (600 µg/kg body weight) intra gastrically dissolved in distilled water daily for 45 days [14].

At the end of the experimental period, the rats were deprived of food overnight, anesthetized intramuscularly using ketamine (24 mg/ kg body weight) and sacrificed by cervical decapitation. The blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3:1) for the estimation of plasma glucose, insulin, lipid peroxidation and non-enzymatic antioxidants.

Biochemical Estimations

Plasma glucose was estimated using a commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) by the method of Trinder (Trinder, 1969). Plasma insulin was assayed by ELISA kit (Boehringer-Mannheim Kit, Mannheim, Germany). The levels of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH), vitamins C and E, and reduced glutathione (GSH) were estimated in the plasma by standard methods (Fraga *et al.*, 1988; Jiang *et al.*, 1992; Omaye *et al.*, 1979; Baker *et al.*, 1980; Ellman, 1959).

Statistical Analysis

Data presented as means $\pm$ standard deviation (S.D.) and subjected to statistical significance were evaluated by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software package version 16.0 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan's Multiple Range Test (DMRT). Values

are considered statistically significant when $P < 0.05$.

3. Results

Table 1 shows the levels of plasma glucose and plasma insulin in normal control and experimental rats. In STZ-induced diabetic control groups, the level of plasma glucose was significantly ($P < 0.05$) increased and the plasma insulin level was significantly ($P < 0.05$) decreased as compared to normal control group. On the other hand, administration of tyrosol daily for a period of 45 days was found to lower the plasma glucose and increase the insulin level significantly ($P < 0.05$) in a dose dependent manner when compared with STZ-induced diabetic control groups. Tyrosol (20 mg/kg body weight) showed more pronounced effect than the other two doses (5 and 10 mg/kg body weight). Based on these data, the effective dose was fixed as 20 mg/kg body weight and used for further biochemical parameters such as lipid peroxidation and non-enzymatic antioxidant system.

There was a significant ($P < 0.05$) increase in the levels of plasma TBARS and LOOH in STZ-induced diabetic control rats compared to normal control rats. Administration of tyrosol and glibenclamide to STZ-induced diabetic rats revealed a significant ($P < 0.05$) reduction of TBARS and LOOH in the plasma when compared to STZ-induced diabetic control rats (Figure - 1 and 2).

The levels of non-enzymatic antioxidants such as vitamin-C, vitamin-E and GSH were significantly ($P < 0.05$) decreased in the plasma of STZ-induced diabetic control rats compared to normal control rats. Treatment with tyrosol and glibenclamide to STZ-induced diabetic rats restored the levels of the above mentioned non-enzymatic antioxidants in the plasma (Figure - 3 and 4).

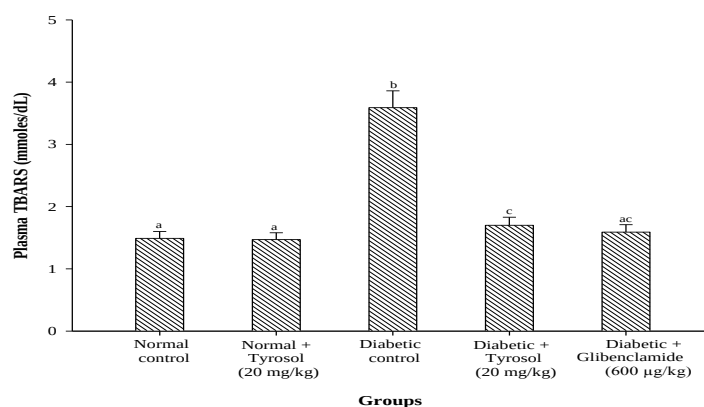
For all the biochemical parameters evaluated, tyrosol treatment to normal rats (Group II) did not show any effect as compared to normal control group (Group I).



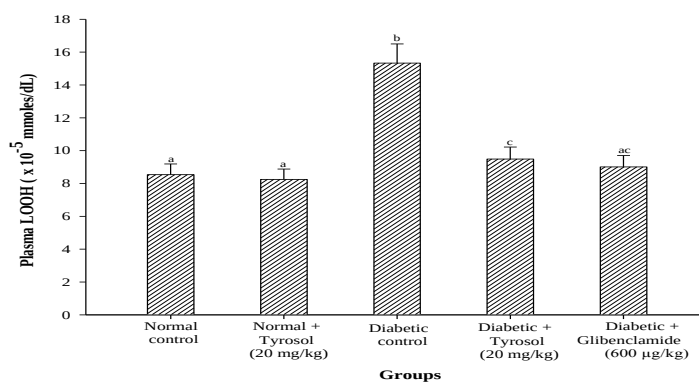
Table – 1: Changes in the levels of plasma glucose and insulin.

Groups	Glucose (mg/dL)	Insulin (μ U/ml)
Normal control	86.70 $\pm$ 6.60 ^a	16.09 $\pm$ 1.23 ^a
Normal +Tyrosol (20 mg/kg bw)	87.38 $\pm$ 6.69 ^a	16.34 $\pm$ 1.25 ^a
Diabetic control	271.39 $\pm$ 20.66 ^b	6.21 $\pm$ 0.47 ^b
Diabetic + Tyrosol (5mg/kg bw)	215.39 $\pm$ 16.49 ^c	8.23 $\pm$ 0.63 ^c
Diabetic + Tyrosol (10mg/kg bw)	185.85 $\pm$ 14.23 ^d	11.10 $\pm$ 0.85 ^d
Diabetic + Tyrosol (20mg/kg bw)	117.92 $\pm$ 9.03 ^e	12.93 $\pm$ 0.99 ^e
Diabetic + Glibenclamide (600 μ g/ kg bw)	102.61 $\pm$ 7.85 ^f	14.70 $\pm$ 1.12 ^f

Each value is mean $\pm$ S.D. for six rats in each group. In each group, means with different superscript letter (a–f) differ significantly at $p < 0.05$ (DMRT).

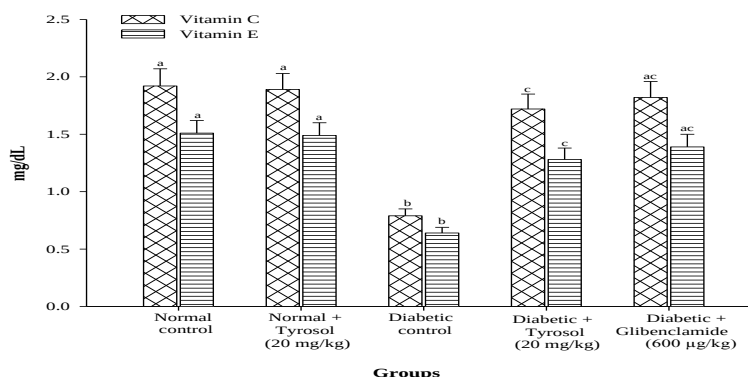


Each column is mean $\pm$ S.D. for six rats in each group. In each bar, means with different letter (a–c) differ significantly at $P < 0.05$ (DMRT)

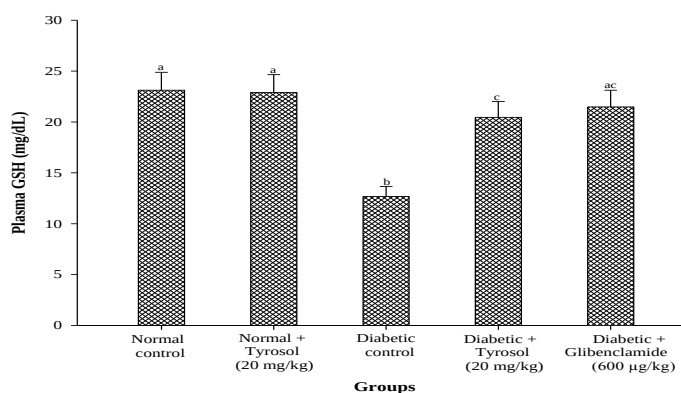
Figure - 1: Changes in the levels of plasma TBARS.

Each column is mean $\pm$ S.D. for six rats in each group. In each bar, means with different letter (a–c) differ significantly at $P < 0.05$ (DMRT)



Figure - 2: Changes in the levels of plasma LOOH

Each column is mean $\pm$ S.D. for six rats in each group. In each bar, means with different letter (a–c) differ significantly at $P < 0.05$ (DMRT)

Figure - 3: Changes in the levels of plasma vitamin-C and vitamin-E

Each column is mean $\pm$ S.D. for six rats in each group. In each bar, means with different letter (a–c) differ significantly at $P < 0.05$ (DMRT)

Figure - 4: Changes in the levels of plasma GSH

4. DISCUSSION

In our study, we observed a significant increase in plasma glucose and a significant decrease in plasma insulin levels in STZ-induced diabetic control rats. Intraperitoneal administration of STZ (40 mg/kg body weight) causes partial pancreatic β -cell dysfunction, which decreases the synthesis and secretion of insulin, thereby producing hyperglycaemia (Stephen Irudayaraj *et al.*, 2012; Punithavathi *et al.*, 2011; Sheikh *et al.*, 2015). The increased levels of plasma glucose and decreased levels of insulin were restored

to near normal by tyrosol treatment in a dose dependent manner and those results were similar to that of glibenclamide. Possibly tyrosol enhanced the insulin secretion from remnant pancreatic cells, which in turn enhance glucose utilization by peripheral tissues of STZ-induced diabetic rats either by promoting glucose uptake and metabolism, or by inhibiting hepatic gluconeogenesis and decreased blood glucose levels.

Lipid peroxidation is one of the characteristic features of diabetes mellitus. Our



study revealed a significant increase of plasma TBARS and LOOH levels in diabetic rats, which may be due to oxidative stress. The increased levels of plasma lipid peroxidation products observed in STZ-induced diabetes is generally thought to be due to pathological changes in tissues that increase the production and liberation of lipid peroxides into the circulation. In this context, diabetic patients also showed an increase in the levels of lipid peroxidation products such as TBARS and LOOH in the circulation (Likidilid, 2010). The decreased levels of plasma TBARS and LOOH observed in tyrosol and glibenclamide treated STZ-induced diabetic rats is due to its antilipid peroxidation effect. Thus, tyrosol scavenges excessive free radicals produced by STZ and reduces oxidative stress, thereby protecting the tissues from the deleterious effects of lipid peroxidation.

The non-enzymatic antioxidants such as vitamin-C, vitamin-E and GSH play a vital role in protecting the cells from hyperglycemia mediated oxidative stress. In this study, the STZ-induced diabetic rats showed a significant depletion of non-enzymatic antioxidants (vitamin-C, vitamin-E and GSH) in the plasma. This could be due to increased free radical production in DM. These findings are in concordance with a previous study (Umamaheswari and Stanely Mainzen Prince, 2007). Treatment with tyrosol and glibenclamide increased the levels of plasma vitamin C, vitamin E, and GSH in STZ-induced diabetic rats. This effect shows the antioxidant potential of tyrosol against hyperglycemia caused by excessive free radicals in STZ-induced diabetic rats.

In conclusion, tyrosol treatment restored altered levels of plasma glucose, insulin, lipid peroxidation and non-enzymatic antioxidants in STZ-induced diabetic rats. Our study also revealed that tyrosol ameliorated altered lipid peroxidation and non-enzymatic antioxidants in the STZ-induced diabetic rats from oxidative stress associated complications, by virtue of its antihyperglycaemic and antioxidant effects.

Acknowledgement

We thank the University Grants Commission (UGC), New Delhi, India for funding support in the form of research fellow under Research Fellowship in Science for Meritorious Students (RFSMS) Scheme (F4-1/2006(BSR)/7-10/2007(BSR)) to Mr. R. Chandramohan.

5. REFERENCES

- 1) Ahn EY, Jiang Y, Zhang Y, Son EM, You S, Kang SW, Park JS, Jung JH, Lee BJ, Kim DK. Cytotoxicity of p-tyrosol and its derivatives may correlate with the inhibition of DNA replication initiation. *Oncol Rep* 2008; 19:527–534.
- 2) Baker H, Frank O, Angelis B, Feingold S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr Rep Int* 1980; 21: 531– 536.
- 3) Bu Y, Rho S, Kim J, Kim MY, Lee DH, Kim SY, Choi H, Kim H. Neuroprotective effect of tyrosol on transient focal cerebral ischemia in rats. *Neurosci Lett* 2007; 414: 218–221.
- 4) Calcutt NA, Cooper ME, Tim S, Kern TS, Schmidt AM. Therapies for hyperglycaemia-induced diabetic complications: from animal models to clinical trials. *Nature Rev Drug Discov* 2009; 8:417–429.
- 5) Casas EM, Albadalejo MF, Planells MIC, Colomer MF, Lamuela Ravento's RM, Fornell RT. Tyrosol Bioavailability in Humans after Ingestion of Virgin Olive Oil. *Clin Chem* 2001; 47:341–343.
- 6) Chernyshov GA, Plotnikov MB, Smol'iakova VI, Krasnov EA. Therapeutic effect of p-tyrosol on myocardial electric instability induced by coronary occlusion. *Eksp Klin Farmakol* 2007; 70:23–25 (Article in Russian).
- 7) Dewapriya P, Himaya SW, Li YX, Kim SK. Tyrosol exerts a protective effect against dopaminergic neuronal cell death in in vitro model of Parkinson's disease. *Food Chem* 2013; 141:1147–1157.
- 8) Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82:70–77.



- 9) Fraga CG, Leibowitz BE, Toppel AL. Lipid peroxidation measured as TBARS in tissue slices: characterization and comparison with homogenates and microsomes. *J Free Rad Biol Med* 1988; 4:155–161.
- 10) Giovannini C, Straface E, Modesti D, Coni E, Cantafora A, De Vincenzi M, Malorni W, Masella R. Tyrosol, the major olive oil biophenol, protects against oxidized-LDL-induced injury in Caco-2 cells. *J Nutr* 1999; 129:1269–1277.
- 11) IDF, 2013. Diabetes Atlas, 6th ed, <http://www.idf.org/diabetesatlas/6e/the-globalburden>.
- 12) Jiang ZY, Hunt JV, Wolff SP. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. *Anal Biochem* 1992; 202: 384–389.
- 13) Likidilid A, Patchanans N, Peerapatdit T, Sriratanasathavorn C. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients. *J Med Assoc Thai* 2010; 93:682–693.
- 14) Miller NJ, Castelluccio C, Tijburg L, Rice-Evans C. The antioxidant properties of theaflavins and their gallate esters-radical scavengers or metal chelators? *FEBS Lett* 1996; 392: 40–44.
- 15) Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluid. *Methods Enzymol* 1979; 62:3–11.
- 16) Panossian A, Nikoyan N, Ohanyan N, Hovhannisyan A, Abrahamyan H, Gabrielyan E, Wikman G. Comparative study of Rhodiola preparations on behavioral despair of rats. *Phytomedicine* 2008; 15:84–91.
- 17) Posuwan J, Prangthip P, Leardkamolkarn V, Yamborisut U, Surasiang R, Charoensiri R, Kongkachuichai R. Long-term supplementation of high pigmented rice bran oil (*Oryza sativa* L.) on amelioration of oxidative stress and histological changes in streptozotocin-induced diabetic rats fed a high fat diet; Riceberry bran oil. *Food Chem* 2013; 138:501–508.
- 18) Punithavathi VR, Stanely Mainzen Prince P, Kumar R, Selvakumari J. Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. *Euro J Pharmacol* 2011; 650:465–471.
- 19) Rudge MV, Damasceno DC, Volpato GT, Almeida FC, Calderon IM, Lemonica IP. Effect of Ginkgo biloba on the reproductive outcome and oxidative stress biomarkers of streptozotocin-induced diabetic rats. *Braz J Med Biol Res* 2007; 40:1095–1099.
- 20) Selvaraj N, Bobby Z, Koner BC, Das AK. Reassessing the increased glycation of hemoglobin in nondiabetic chronic renal failure patients: a hypothesis on the role of lipid peroxides. *Clin Chim Acta* 2005; 360:108–113.
- 21) Sheikh BA, Pari L, Rathinam A, Chandramohan R. Trans-anethole, a terpenoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats. *Biochimie* 2015; 112:57–65.
- 22) Shrilatha B, Muralidhara. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. *Reprod Toxicol* 2007; 23:578–587.
- 23) Stephen Irudayaraj S, Sunil C, Duraipandiyar V, Ignacimuthu S. Antidiabetic and antioxidant activities of *Toddalia asiatica*(L.) Lam. leaves in streptozotocin induced diabetic rats. *J Ethnopharmacol* 2012; 143:515–523.
- 24) St-Laurent-Thibault C, Arseneault M, Longpre F, Ramassamy C. Tyrosol and hydroxytyrosol, two main components of olive oil, protect N2a cells against amyloid-b-induced toxicity. Involvement of the NF-kB signaling. *Curr Alzheimer Res* 2011; 8:543–551.



- 25) Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; 6:24-27.
- 26) Ugarte M, Brown M, Hollywood KA, Cooper GJ, Bishop PN, Dunn WB. Metabolomic analysis of rat serum in streptozotocin-induced diabetes and after treatment with oral triethylenetetramine (TETA). *Genome Med* 2012; 4:35.
- Umamaheswari S, Stanely Mainzen Prince P. Antihyperglycaemic effect of 'Ilogen-Excel'. An ayurvedic herbal formulation in streptozotocin induced diabetes mellitus. *Acta Pol Pharm* 2007; 64:53-61.

