Indian Journal of Clinical Biochemistry, 2007 / 22 (1) 77-83

ANTIHYPERGLYCEMIC AND ANTILIPIDPEROXIDATIVE EFFECTS OF *TEPHROSIA PURPUREA* SEED EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

P. Pavana, S.Sethupathy* and S. Manoharan

Department of Biochemistry, Faculty of Science and
**Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamilnadu*

ABSTRACT

The aim of the present study was to evaluate the antihyperglycemic and antilipidperoxidative effects of ethanolic seed extract of Tephrosia purpurea *(TpEt) in streptozotocin induced diabetic rats. Hyperglycemia associated with an altered hexokinase and glucose 6 phosphatase activities, elevated lipid peroxidation, disturbed enzymatic and non-enzymatic antioxidants status were observed in streptozotocin induced diabetic rats. Oral administration of "TpEt" at a dose of 300mg/kg bw showed significant antihyperglcemic and antilipidperoxidative effects as well as increased the activities of enzymatic antioxidants and levels of non enzymatic antioxidants. We also noticed that the antihyperglycemic effect of plant drug (TpEt) was comparable to that of the reference drug glibenclamide. Our results clearly indicate that "TpEt" has potent antihyperglycemic and antilipidperoxidative effects in streptozotocin induced diabetic rats and therefore further studies are warranted to isolate and characterize the bioactive antidiabetic principles from "TpEt".*

KEY WORDS

Streptozotocin, Tephrosia purpurea, *Antioxidants, Lipid peroxidation.*

INTRODUCTION

Diabetes mellitus, a leading non communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world (1). The World Health Organization (WHO) reported that 300 million peoples would suffer from diabetes mellitus by the year 2025 (2). India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that diabetes affects approximately 57 million people by the year 2025 in India (3). Diabetes mellitus is characterized by an increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective secretion of insulin. These metabolic disturbances result in acute and long-term diabetic complications, which are responsible for premature death and disability (4).

Address for Correspondence :

Dr. S. Manoharan
Senior Lecturer, Dept of Biochemistry,
Faculty of Science, Annamalai University,
Annamalai Nagar -608002 Tamilnadu, India.
E-mail : manshisak@yahoo.com

Several studies have proposed the mechanism for the role of free radicals in the pathogenesis of various diseases including diabetes mellitus (5). However, an array of non-enzymatic antioxidants [vitamin E, vitamin C and reduced glutathione (GSH)] and enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx)] defense mechanism are involved in the protection of free radicals induced oxidative damage. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins (6). Recent reports indicate that diabetic complications are associated with overproduction of free radicals and accumulation of lipid peroxidation by-products (7). Enhanced oxidative stress has been well documented in both experimental and human diabetes mellitus (8).

Many herbal medicines as single agents or in different oral formulations have been recommended for diabetes mellitus due to the fact that they are less toxic than oral hypoglycemic agents such as sulfonylureas, metformin etc (9,10). *Tephrosia purpurea* (Linn.) pers, is a polymorphic, much-branched sub-erect perennial herb, popularly known as "Sarapunkha" in Sanskrit, "Purpule tephrosia" in English and "Kattukkolincai"

in Tamil. Different parts of *T.purpurea* have been used for diabetes mellitus in Ayurvedic and Siddha medicine. It is considered beneficial for liver, spleen and kidney disorders (11, 12). Experimental studies have demonstrated its antiulcer and hepatoprotective effects (13, 14). To our best knowledge, there were no scientific data on antihyperglycemic and antilipid peroxidative effects of *T. Purpurea* seeds in experimental diabetes mellitus. Hence, the present study was designed to evaluate antihyperglycemic and antilipidperoxidative effects of *T.purpurea* seeds in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals : Streptozotocin was purchased from Sigma Aldrich Chemicals, Pvt., Ltd., Bangalore. All other chemicals and reagents used were of analytical grade.

Plant material : Seeds of *Tephrosia purpurea* were collected in and around chidambaram, Tamilnadu and it was botanically authenticated. A voucher specimen (AU05102) was deposited in the Department of Botany, Annamalai University, Annamalainagar, Tamilnadu.

Preparation of plant extract : The ethanolic extract of *Tephrosia purpurea* seeds was prepared according to the method of Hossain et al (15). 500 g of *Tephrosia purpurea* seeds were dried, powdered and then soaked in 1500 ml of 95% of ethanol over night. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 hrs and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapour at 40-50^{0}C under reduced pressure. A 20% semisolid light greenish yellow material obtained was stored at 0-4^{0}C until used. A known volume of the residual extract is suspended in distilled water and was orally administered to the animals during the experimental period.

Animals : Albino wistar male rats 7 to 8 weeks old and weighing150-200g were used for the present study. The animals were obtained from Central animal house, Rajah Muthiah Institute of Health Sciences, Annamalai University, India and were maintained in the central animal house with 12 h light and 12 h dark cycles. The animals were randomized into control and experimental groups and housed 4 or 5 in polypropylene cages. Standard pellets obtained from Mysore Snack Feed Ltd, Mysore, India, were used as a basal diet during the experiment. The control and experimental animals were provided food and drinking water *ad libitum*.

Induction of diabetes mellitus : Diabetes mellitus was induced in wistar rats by single intraperitoneal injection of streptozotocin (50mg/kg) dissolved in 0.1M-citrate buffer (pH 4.5) after overnight fasting for 12 h [16]. The diabetes was assessed by determining the blood glucose concentration within 48 hours after injection of streptozotocin. The rats with blood glucose level above 250 mg/dl were selected for the experimental studies

Experimental design : In the experiment a total number of 24 rats (18 diabetic rats, 6 normal rats) were used. The rats were divided into 4 groups of six each.

Group I : Control rats
Group II : Diabetic control (streptozotocin 50mg/kg bw i.p)
Group III : Diabetic rats receiving “TpEt” (300 mg/kg bw orally)
Group IV : Diabetic rats receiving glibenclamide (600 μg/kg bw orally)

After the experimental period, all animals were sacrificed by cervical dislocation and biochemical studies were conducted on blood, plasma, erythrocytes, erythrocyte membranes and liver of control and experimental animals in each group.

Isolation of the Erythrocyte Membranes : The erythrocyte membrane was isolated according to the procedure of Dodge *et al.,* (17) with a change in buffer according to Quist (18). The packed cells remaining after the removal of plasma were washed thrice with isotonic tris-HCl buffer, pH 7.4. Hemolysis was performed by pipetting out the washed suspension of red blood cells into polypropylene centrifuge tubes containing hypotonic tris-HCl buffer, pH 7.2. Ghosts were sedimented by using a high-speed refrigerated centrifuge at 20,000g for 40 minutes. The hemolysate was decanted carefully and the ghost button was resuspended by swirling. Sufficient buffer of the same strength was added to reconstitute the original volume. The ratio of the cells to washing solution was approximately 1:3 by volume. The same procedure was repeated for three times until membrane become colorless or pale pink. The pellets of erythrocyte were resuspended in a known volume of 0.1 M tris-HCl buffer, pH 7.2. Aliquots from this preparation were used for biochemical estimation.

Preparation of Erythrocyte Haemolysate : After plasma separation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. A known volume of erythrocytes was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 10,000 rpm for 15 min at 20 °C.

Biochemical estimation : Blood glucose was determined by the method of Sasaki *et al*., (19) using O-toluidine reagent. Hexokinase activity was assayed by the method of Brandstrup *et al* (20). The method is based on the phosphorylation of glucose by the enzyme hexokinase (tissue homogenate) and the residual glucose in the supernatant was determined by the method of Sasaki *et al* (19). Glucose-6-phosphatase was assayed according to the method of Koida and Oda (21). The method is based on the estimation of inorganic phosphorus liberated from the glucose-6-phosphate present in the incubation mixture (glucose-6-phosphate, tissue homogenate and malic acid buffer). The liberated inorganic phosphorus was estimated by the method of Fiske and Subbarow (22).

TBARS in plasma was assayed by the method of Yagi (23). Plasma was deproteinised with 10% phosphotungstic acid and the precipitate was treated with thiobarbituric acid at 90^{o}C for 1 hour. After cooling, 5.0ml of n-butanol was added and the mixture was shaken vigorously and centrifuged at 1000g for 15 minutes. The pink colour formed gives a measure of the thiobarbituric acid reactive substances (TBARS). TBARS in erythrocytes and erythrocyte membranes were assayed according to the method of Donnan (24). The erythrocytes and erythrocyte membranes were deproteinized with 10% TCA and then treated with TBA. The above mixture was heated in a boiling water bath for 15 minutes. It was cooled at room temperature and pink color that developed was measured at 535nm.

The activity of glutathione peroxidase was estimated according to the method of Rotruck *et al* (25). The enzyme preparation was allowed to react with H_2O_2 in the presence of reduced glutathione for a specified time period, and then the remaining reduced glutathione content was assayed by the method of Beutler and Kelley (26). Superoxide dismutase (SOD) activity was assayed by the method of Kakkar *et al* (27). The assay of superoxide dismutase was based on the inhibition of formation of NADH-phenazine methosulphate nitroblue tetrazolium formazan. The colour formed at the end of the reaction was extracted into butanol layer and measured at 520nm. Catalase activity was assayed using the method of Sinha (28). The method is based on the utilization of H_2O_2 by the enzyme. The color developed was read at 620nm.

Reduced glutathione was measured according to the method of Beutler and Kelley (26). The technique involved in protein precipitation by meta phosphoric acid and spectrophotometric assay at 412nnm of the yellow derivative obtained by the reaction of supernatant with 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB). Vitamin E was estimated by the method of Desai (29) based on the classical Emmerie Engle reaction. The lipid residue obtained using redistilled ethanol and petroleum ether was redissolved in absolute ethanol. To this solution, ferric chloride, orthophosphoric acid and bathophenanthroline reagents were added. Vitamin E present in the lipid residue reduces ferric ion to ferrous ions and forms a pink colored complex with bathophenanthroline orthophosphoric acid. Absorption due to the pink complex was measured at 536nm. Ascorbic acid level was estimated by the method of Omaye *et al* (30). Copper to form dehydroascorbic acid and diketoglutaric acid oxidizes ascorbic acid. These products when treated with 2, 4-dinitrophenylhydrazine (DNPH) form the derivatives bis-2,4-dinitrophenylhydrazone which undergoes rearrangement to form a product with an absorption maximum at 520nm.

Statistical Analysis : The data are expressed as mean ± SD. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Student's t test. The results were considered statistically significant if the p values were 0.05 or less.

RESULTS

Table 1 shows the level of blood glucose in control and experimental animals in each group. The level of blood glucose was significantly increased in streptozotocin alone treated rats (Group II) as compared to control animals (Group I). However, the level of blood glucose was returned to near normal concentrations in diabetic rats treated with "TpEt" and glibenclamide. "TpEt" showed comparable effect to that of glibenclamide.

Table 1 : Blood Glucose level in control and experimental animals in each group

Groups	Blood Glucose (mg/dl)
Control	78.5±6.8
Diabetic control	283.2±14.2^{a}
Diabetic + alc TpEt (300 mg/kg bw)	105.6± 8.5^{ab}
Diabetic + glibenclamide (600 μg/kg bw)	115.3±10.5^{abc}

Values are given as mean ± SD (n= 6 rats).
a- significantly different from control animals $^{a}p<0.001$
b- significantly different from diabetic control $^{b}p<0.001$
c-as compared to diabetic +alc TpEt treated rats ^{c}p-not significant

Table 2 indicates the activities of hexokinase and glucose-6-phosphatase in the liver of control and experimental animals in each group. A significant decrease in hexokinase and increase in glucose-6-phosphatase activities were noticed in the liver of diabetic animals as compared to control animals.

Oral administration of "TpEt" to diabetic animals revert back the enzyme activities to near normal concentrations.

Table 2 : Activities of hexokinase and glucose-6-phosphatase in the liver of control and experimental animals in each group

Groups	Hexokinase (U* /mg protein)	Glucose-6-phosphatase (U ** / mg protein)
Normal	0.27±0.03	2.65 ± 0.08
Diabetic control	0.12±0.02^{a}	3.85± 0.12^{a}
Diabetic + alc TpEt (300mg/kg bw)	0.23 ±0.02^{b}	3.04 ± 0.11^{a*b}
Diabetic+glibenclamide (600μg/kg bw)	0.20 ± 0.01$^{a°bc}$	3.28 ± 0.09$^{ab°c}$

Values are given as mean ± SD (n= 6 rats)
a-significantly different from control animals $^{a}p<0.001$, $^{a°}p<0.01$, $^{a*}p<0.05$
b-significantly different from diabetic control $^{b}p<0.001$
c-significantly different from diabetic +alc TpEt treated rats $^{c}p<0.01$, $^{°c}p<0.05$
U*- Micromoles of glucose phosporylated/ h
U** - Micromoles Pi liberated/min

Table 3 shows the level of plasma, erythrocytes and erythrocyte membranes TBARS in control and experimental animals in each group. The levels of TBARS were significantly increased in diabetic animals as compared to control animals. The levels of TBARS were significantly reduced in diabetic animals treated with "TpEt".

Table 4 & 5 show the levels of non-enzymatic antioxidants and activities of enzymatic antioxidants in plasma and erythrocytes respectively in control and experimental animals in each group. The levels of non-enzymatic antioxidants and activities of enzymatic antioxidants were decreased in diabetic animals as compared to control animals. However, oral administration of "TpEt" at a dose of 300mg/kg bw revert back the levels of non-enzymatic antioxidants and activities of enzymatic antioxidants to near normal range in diabetic animals.

Table 3 : Levels of TBARS in plasma, erythrocytes and erythrocyte membranes of control and experimental animals in each group

Groups	Plasma (nmol/ml)	Erythrocyte membranes (nmol/mg protein)	Erythrocytes (pmol/mg Hb)
Normal	1.9 ± 0.18	0.35 ± 0.04	0.65±0. 08
Diabetic control	3.1± 0.21^{a}	0.85 ± 0.07^{a}	0.94±0. 07^{a}
Diabetic + alc TpEt (300mg/kg bw)	2.2± 0.16$^{a°b}$	0.56 ± 0.05^{ab}	0.71±0.05^{b}
Diabetic+ glibenclamide (600μg/kg bw)	2.5± 0.24$^{ab°c}$	0.61 ± 0.03$^{ab°c}$	0.77±0. 06^{a*b}

Values are given as mean ± SD (n= 6 rats)
a-significantly different from control animals $^{a}p<0.001$, $^{a°}p<0.01$, $^{a*}p<0.05$
b-significantly different from diabetic control $^{b}p<0.001$
c-significantly different from diabetic +alc TpEt treated rats $^{°c}p<0.05$

DISCUSSION

Diabetes mellitus is a life threatening metabolic disorder and it is estimated that its annual incidence rate will continue to increase in the future worldwide. Hyperglycemia, the primary clinical manifestation of diabetes mellitus, is associated with the development of micro and macro vascular diabetic complications (31). In the present study, TBARS levels were significantly increased and antioxidants were decreased in

Table 4 : Enzymatic antioxidants and non- enzymatic antioxidants in the plasma of control and experimental animals in each group

Groups	GSHPx (U*/l)	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Reduced Glutathione (mg/dl)
Control	132.6±11.5	2.1±0.10	1.42±0.07	27.4±2.3
Diabetic control	96.5 ± 10.8^{a}	1.2±0.07^{a}	0.85±0.06^{a}	16.5±1.8^{a}
Diabetic + alc TpEt (300 mg/kg bw)	128.3 ± 8.5^{a*b}	1.95±0.08^{a*b}	1.24±0.07^{ab}	23.4±2.1^{b}
Diabetic + glibenclamide (600μg/kg bw)	121.4 ± 7.9$^{ab°c}$	1.85±0.09^{ab}	1.12±0.05$^{ab°c}$	20.3±1.5^{b}

Values are given mean ± SD (n=6 rats)
^{U*}Micromoles of glutathione utilized / min
a-significantly different from control animals $^{a}p<0.001$, $^{a*}p<0.05$
b-significantly different from diabetic control $^{b}p<0.001$
c-significantly different from diabetic +alc TpEt treated rats $^{°c}p<0.05$

Table 5 : Enzymatic antioxidants and non- enzymatic antioxidants in erythrocytes and erythrocyte membranes of control and experimental animals in each group

Groups	SOD (U^a/mg Hb)	Catalase (U^b/mg Hb)	GSHPx (U^c/g Hb)	Vitamin E in erythrocyte membranes (μg/ mg protein)	Reduced Glutathione (mg/dl)
Control	5.52 ± 0.63	123.5 ± 12.8	13.2±1.56	1.05±0.09	38.5±2.9
Diabetic control	3.59 ± 0.29^a	82.16 ± 7.8^a	7.5± 1.01^a	0.62±0.06^a	24.3±2.1^a
Diabetic + alc TpEt (300 mg/kg bw)	4.85 ±0. 32^{a*b}	114.2±10.4^b	10.2±0 .82^{a*b}	0.86±0.06$^{a°b}$	34.2±3.1^{a*b}
Diabetic + glibenclamide (600μg/kg bw)	4.55 ±0 .41$^{a°bc}$	105.1 ± 9.7^{a*bc}	9.2 ± 0.72$^{a°bc}$	0.81±0.04^{abc}	32.5±3.4$^{a°bc}$

Values are given mean ± SD (n=6 rats)
a-significantly different from control animals $^a p<0.001$, $^{a°} p<0.01$, $^{a*} p<0.05$
b-significantly different from diabetic control $^b p<0.001$
c-as compared to diabetic +alc TpEt treated rats ^{c}p-not significant
U^a- The amount of enzyme required to inhibit 50% NBT reduction, U^b- Micromoles of H_2O_2 utilized/ min, U^c- Micromoles of glutathione utilized / min

diabetic rats. Reactive oxygen species induced oxidative damage has been implicated in the pathogenesis of several disorders including diabetes mellitus (32). Streptozotocin damages pancreatic b–cells possibly by generating excess reactive oxygen species, and thus widely used for the induction of experimental diabetes mellitus. Streptozotocin generated lipidperoxidation and DNA breaks in pancreatic islet cells have been demonstrated (33). Prakasam *et al* (34) have reported an elevated lipid peroxidation and lowered antioxidants in streptozotocin induced diabetes mellitus.

Measurement of plasma TBARS help to assess the extent of tissue damage (35). Elevated plasma TBARS observed in the diabetic rats can therefore be related to overproduction of lipid peroxidation byproducts and diffusion from damaged pancreatic tissues. The major pathological consequence of free radical induced membrane lipid peroxidation includes increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity (36). Hunt *et al* (37) reported that glucose oxidation in the presence of transition metals result in excessive generations of reactive oxygen species, which in turn affect biomembrane structure and function by mediating lipid peroxidation process. Enhanced TBARS and declined antioxidants observed in the erythrocytes of diabetic rats can therefore be attributed to increased biomembrane lipidperoxidation process and thereby contributing to alterations in antioxidants status.

Vitamin E is one of the most important free radical scavenging chain-breaking antioxidant within biomembrane (38). Reduced glutathione, a major endogenous antioxidant, plays a crucial role in the antioxidant defense (39). Vitamin C, a major extra cellular non-enzymatic antioxidant, has crucial role in scavenging several reactive oxygen species. Enzymatic antioxidants (SOD, CAT, GSHPx) form the first line of antioxidant defense mechanism to protect the organism from ROS mediated oxidative damage (40). Several studies have demonstrated lowered non-enzymatic antioxidant levels and enzymatic antioxidant activities in streptozotocin induced diabetic rats (41, 42). Our results lend credibility to these observations.

In the present study, orally administered “TpEt” to diabetic rats at dose of 300 mg/kg bw for 45 days showed significant antihyperglycemic and antilipid peroxidative effects as well as improved antioxidant defense mechanism. The antihyperglycemic activity of “TpEt” is probably due to stimulation of insulin secretion from remnant pancreatic β– cells, which in turn enhance glucose utilization by peripheral tissues of diabetic rats. The observed increase in hexokinase activity and decrease in glucose-6-phosphatase activity in diabetic rats treated with “TpEt” suggest its stimulatory effects on glycolysis and inhibitory action on gluconeogenesis in diabetes mellitus. The observed increase in antioxidant status and decline in TBARS concentration in “TpEt” treated diabetic rats suggests its potent antilipidperoxidative and antioxidative effects. Furthermore the plant drug was found to be as effective as that of the reference drug glibenclamide. Further studies are therefore needed to isolate and characterize the bioactive antidiabetic principles from T.*purpurea* seeds.

REFERENCES

1. Zimmet PZ. Diabetes epidemiology as a tool to trigger diabetic research and care. Diabetologia 1999; 42: 499-518.
2. Pradeepa R, Mohan V. The changing of the diabetes epidemic implications for India. Indian J Med Res 2002; 116: 121-32.
3. Aravind K, Pradeepa R, Deepa R. Diabetes and coronary artery disease. Indian J Med Res 2002; 116: 163-76.
4. De Leiva A, Lefebvre P, Nerup J. European dimension of diabetes research. Dibetologia 1995; 39: 5-11.
5. Paolisso G, De Amore A, Di Maro G, D Onofrio F. Evidence for a relationship between free radicals and insulin action in the elderly. Metabolism 1993; 42: 659-63.
6. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress and antioxidants. A Review. J Biochem Mol Toxicol 2003; 17: 24-38.
7. Palanduz S, Ademoglu E, Gokkusu C. Plasma antioxidants and type 2 diabetes mellitus. Pharmacology 2001; 109: 309-18.
8. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40: 405-412.
9. Ponnachan PTC, Paulose CS, Panikar KR. Effect of leaf extract of Aegle marmelose in diabetic rats. Indian J Exp Biol 1993; 31: 345-7.
10. Chattopadhyay RR. Hypoglycemic effect of Ocimum sanctum leaf extract in normal and streptozotocin diabetic rats. Indian J Exp Biol 1993; 31: 891-3.
11. Kritikar KR, Basu BD. Indian medicinal plants. India: Lalif Mohan Basu, 1956.
12. Joshi SG. Medicinal plants. New Delhi: Oxford and IBH publishing Co. Pvt. Ltd, 2000.
13. Despande SS, Shah GB. Antiulcer activity of Tephrosia purpurea in rats. Inidan J Pharmocol 2003; 35:168-172.
14. Ramamurthy SM, Srinivasan M. Hepatoprotective effect of Tephrosia purpurea in experimental animals. Indian J Pharmacol 1993; 25: 34-8.
15. Hossain MZ, Shibib BA, Rahman R. Hypoglycemic effect of Coccinia indica. Inhibition of key gluconeogenic enzyme, glucose-6-phosphatase. Indian J Exp Biol 1992; 10: 418-20.
16. Chang KJ. Effect of taurine and beta alanine on morphological changes of pancreas in streptozotocin induced diabetic rats. Adv Exp Med Biol 2000; 483: 571-7.
17. Dodge JF, Mitchell G, Hanahan DJ. The preparation and chemical characterization of haemoglobin free ghosts of human red blood cells. Arch Biochem Biophys 1968; 110: 119-30.
18. Quist E E. Regulation of erythrocyte membrane shape by Ca^{2+}. Biochem Biophys Res commun 1980; 92: 631-7.
19. Sasaki T, Matsy S, Sonae A. Effect of acetic acid concentration on the color reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho kagaku 1972; 1: 346-53.
20. Brandstrup N, Kirk JE, Bruni C. Determination of hexokinase in tissues. J Gerontol 1957; 12: 166-171.
21. Koida H, Oda T. Pathological occurrence of glucose-6-phosphatase in liver disease. Clin Chim Acta 1959; 4: 554-61.
22. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem 1925; 66: 375-400.
23. Yagi K. Lipid peroxides and human disease. Chem Phys Lipids 1978; 45: 337-51.
24. Donan SK. Thiobarbituric acid test applied to tissues from rats treated in various ways. J Biol Chem 1950; 182: 415-9.
25. Rotruck JT, Pope AL, Ganther HE, Swanson AB. Selenium Biochemical roles as a component of glutathione peroxidase. Science 1984; 179: 588-90.
26. Beutler E, Kelly BM. The effect of sodium nitrate on RBC glutathione. Experienta 1963; 19: 96-7.
27. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 1984; 21: 130-32.
28. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-94.
29. Desai I D. Vitamin E analysis methods for animal tissues. In: Feischer S, Packer L, eds. Methods Enzymol. Academic press, New York: 1984; 105: 138-45.
30. Omaye ST, Turnbull TD, Sauberlich HE. Selected method for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCromic DB, Wright DL, eds. Methods Enzymol. Academic press, New York 1979; 62: 3-11.
31. Brownlee M, Cerami A. The biochemistry of the complications of diabetes mellitus. Annu Rev Biochem 1981; 50: 385-432.
32. Slater TF. Free radical mechanism in tissue injury. J Biochem 1984; 22: 1-15.
33. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetognic action of streptozotocin. Cancer Chemother Rep 1963; 29: 91-8.
34. Prakasam A, Sethupathy S, Pugalendi KV. Erythrocyte redox status in streptozotocin diabetic rats. Effect of Casearia esculenta root extract. Pharmazie 2003; 58: 920-24.
35. Gutteridge JMC. Lipid peroxidation and antioxidant as biomarkers of tissue damage. Clin Chem 1995; 14: 1819-28.

36. Kolanjiappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocytes lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin Chim Acta 2002; 326: 143-9.

37. Hunt JV, Smith CCT, Wolff SP. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 1990; 39: 1420-24.

38. Parks E, Traber MG. Mechanism of vitamin E regulation research over the past decade and focus on the future. Antioxid Redox Signal 2000; 2: 405-12.

39. Anuradha CV, Selvam R. Effect of oral methionine on tissue lipidperoxidation and antioxidants in alloxan induced diabetic rats. J Nutr Biochem 1993; 4: 212-7.

40. Nonaka A, Manabe T. Effect of new synthetic ascorbic acid derivative as a free radical scavenger on the development of acute pancreatitis in mice. Gut 1991; 32: 528-32.

41. Venkateswaran S, Pari L. Antioxidant effect of Phaseolus vulgaris in streptozotocin-induced diabetic rats. Asia-Pacific J Clin Nutr 2002; 11: 206-9.

42. Ananthan R, Latha M, Pari L, Baskar C, Narmatha V. Modulatory effects of Gymnema montanum leaf extract on alloxan induced oxidative stress in wistar rats. Nutrition 2004; 20: 280-85.