Evaluating the effect of vitamin E supplementation in alloxan-induced diabetic rabbits

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Abstract

Objectives: This study evaluated the effectiveness of vitamin E (vit E) supplementation in diabetic rabbits in decreasing their hyperglycemic condition and oxidative stress (O.S.) status. **Materials and Methods:** 10 albino rabbits were randomly selected and made diabetic by administrating Alloxan. Out of 10 diabetic rabbits, five were treated with insulin. Another ten healthy rabbits were selected and grouped into 2, each having five rabbits. Out of four groups (5 rabbits in each group), three groups were treated with vit E (80 mg/kg/day) for six months. The rest of one group of rabbits was kept as healthy control. Their weight, glycaemic parameters like fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycosylated haemoglobin (HbA1c) and oxidative stress parameters like serum vitamin C and malonaldehyde (MDA) levels were estimated. All parameters were tested at the beginning of the study and re-evaluated after six months of treatment. **Result:** There was a significant decrease in FBS and PPBS in all the vit E treated groups, but an insignificant decrease in HbA1c level was found. In all vit E treated groups significant increase in serum vitamin C and a decrease in serum MDA was found. **Conclusion:** So vit E protects against oxidative injury associated with diabetic conditions.

Keywords: Vitamin E, Diabetic Rabbits, Oxidative Stress, Alloxan.

INTRODUCTION

As a rising prevalent condition globally, Diabetes Mellitus (D.M.) is a chronic endocrine disorder characterizing increased blood sugar, causing disturbances in the metabolism of carbohydrates, fat and protein resulting from insulin secretion defect or action defect or both conditions. [1]. The pathogenesis of several diseased conditions involves free radicals (F.R.s). All body cells have adequate quantities of Antioxidants (A.O.s), which protect against free radicals toxic effects, and a scarcity of these A.O.s can damage organs and tissues. One of the FR-mediated oxidative destruction of pancreatic islets of Langerhans leads to D.M. [2 -5].

Alloxan is used experimentally to induce Diabetes in animals as reduced Alloxan causes necrotic death of pancreatic islets. D.M. can only be managed, and many studies reported the usefulness of supplementation of zinc, vitamins E, C, and A, β carotene, dimethyl thiourea, 1α,25-dihydroxy-vitamin-D3, etc., in giving protection against D.M. So adequate intake of vitamin E (vit E) has

been reported as a reasonable means of managing D.M. complications. [6 - 20]. On the contrary, few other studies have shown that vit E intake is ineffective in avoiding O.S. associated with D.M. [7, 21, 22]. As D.M. is a common incidence globally, this study explored the effect of vit E supplementation on glycaemic index, lipid profiles, and oxidative stress markers in experimental D.M. rabbits.

MATERIALS AND METHODS

It was a randomized placebo-controlled study on laboratory animal models in the Department of Pharmacology, IMS & SUM Hospital, Bhubaneswar. The study was approved by Institutional Animal Ethics Committee, IMS & SUM Hospital, Bhubaneswar [004/IAEC/IMS & SH/SOAU]. A total of 20 healthy albino rabbits (weighing range of 1300g-1850g) were kept under observation in the Central Animal House of the institute before starting an experiment for seven days. They were numbered and put in their separate cages. Among ten were made diabetic by administering Alloxan intravenously in rabbits' marginal ear veins with appropriate amounts according to body weight. [23]. The grouping of animals is given in Table 1

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Table 1. Grouping of animals based on their treatment

Group name	Number of animals in the group	Type of rabbits	Supplemented drugs
DIE	5	Diabetic + Insulin	Vitamin-E
DE	5	Diabetics	Vitamin-E
CE	5	Non-diabetics	Vitamin-E
C0	5	Non-diabetics	Normal saline

N.B.: DIE: Diabetic rabbits treated with insulin and vit E simultaneously. DE: Diabetic rabbits only treated with vit E, C.E.: Non-diabetic rabbits treated with vit E, C0: Non-diabetic rabbits neither treated with insulin nor treated with vit E.

Blood samples were collected from the marginal ear with the help of a 24-gauge butterfly catheter needle in a 5 ml sterilized syringe. The glucometer did blood glucose estimation. Glycosylated haemoglobin was estimated by modified ion-exchange high-performance liquid chromatography. [24]. Serum malondialdehyde (MDA) (nm/ml) was estimated manually by Ohkawa H et el., 1979 method using Thiobarbituric acid, sodium sulphate, trichloroacetic acid and sulphuric acid. [25]. Serum ascorbic acid (in mg/dl) is estimated manually by J. George, 2003; using a 2,4-dinitrophenylhydrazine reagent, sulphuric acid and trichloro acetic acid. [26]

RESULTS

Results were expressed as Mean ± Standard Deviation of the Mean. Statistical data analysis was performed using paired 't-tests for intragroup and paired 't-tests, and ANOVA tests were used for the inter-group analysis of rabbits SPSS 20 version software was used for statistical analysis.

Table 2. Weight (grams) of study groups

Group	Weight (g)		
	Before treatment	After treatment	p-value
DIE	1400 ± 93.541	1730 ± 103.682	<0.01, **
D.E.	1400 ± 145.773	1320 ± 130.384	>0.05, ns
C.E.	1390 ± 41.833	2050 ± 35.355	<0.001, ***
C0	1390 ± 65.192	1960 ± 41.833	<0.001, ***

N.B.: In a comparison of weights of rabbits in them before-treatment and after-treatment values, p-value <0.001 is considered very highly significant (***), p<0.01 is highly significant (**), p<0.05 is significant (*), and p>0.05 as not significant (ns). Group names DIE, DE, C.E. and C0 are explained in Table-1.

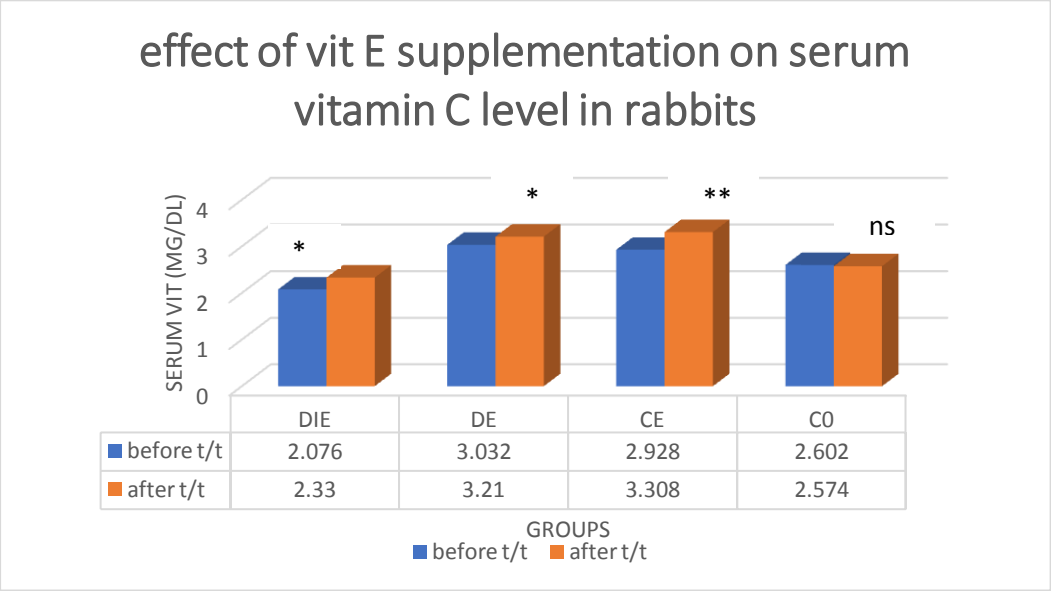
Table 3: Diabetic parameters of study groups

Group	FBS (mg/dl)			PPBS (mg/dl)			HbA1c (%)		
	Before t/t	After t/t	p-value	Before t/t	After t/t	p-value	Before t/t	After t/t	p-value
DIE	195.8 ± 8.871	105.0 ± 6.442	***	225.4 ± 11.631	157.0 ± 8.270	***	7.6 ± 0.343	5.3 ± 0.073	***
D.E.	196.6 ± 11.392	163.0 ± 16.370	**	219.2 ± 16.932	196.8 ± 12.029	**	6.96 ± 0.531	7.12 ± 0.584	ns
C.E.	97.4 ± 6.107	94.6 ± 3.435	ns	122.2 ± 4.024	122.4 ± 4.335	ns	4.86 ± 0.482	4.78 ± 0.408	ns
C0	94.2 ± 10.329	95.0 ± 9.016	ns	118.6 ± 5.639	118.8 ± 5.761	ns	4.66 ± 0.327	4.72 ± 0.443	ns

N.B.: In the comparison of values, p-value <0.001 is considered very highly significant (***), p<0.01 as highly significant (**), p<0.05 as significant (*) and p>0.05 as not significant (ns). Group names DIE, DE, C.E. and C0 are explained in Table-1. t/t: treatment. FBS: fasting blood sugar, PPBS: postprandial blood sugar, HbA1c: glycosylated haemoglobin.

Table 2 shows the weight of all rabbits (in DIE, DE, C.E., and C0 groups) showed a non-significant difference (P>0.05) in them. But after vit E supplementation comparing the weights showed a significant increase (p<0.001) in the rabbits' weight, except in the D.E. group, which showed a non-significant decrease in their weights.

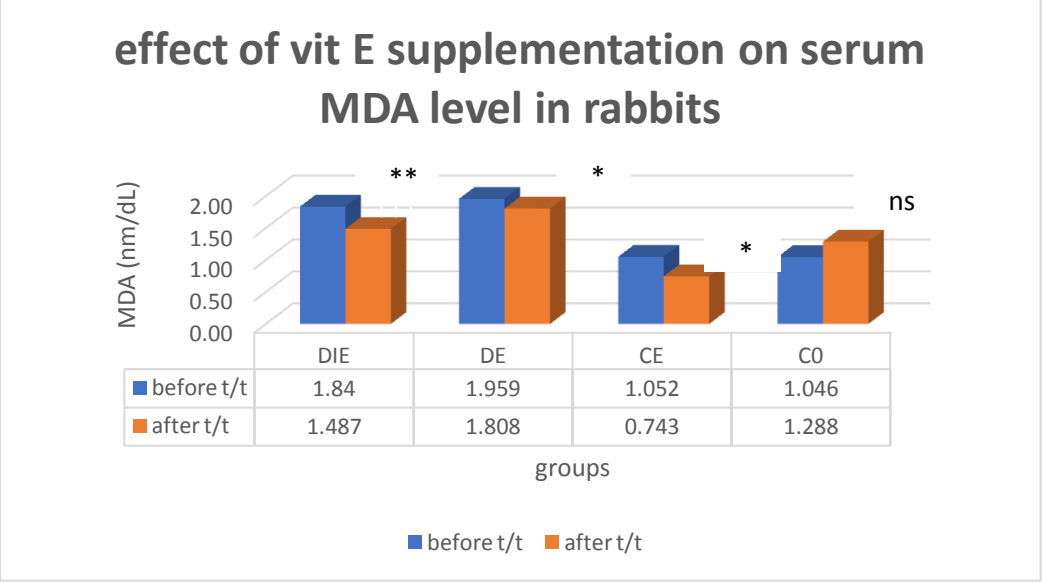
Table 3 shows significant differences in FBS and PPBS in the DIE group (p<0.001) and the D.E. group (p<0.01) when comparing the after-treatment values with the before-treatment values. But there found no significant change in the C.E. group (p>0.05) and the C0 group (p>0.05). The HbA1c shows a significant decrease in the DIE group (p<0.001) but no significant differences between before and after values in D.E., C.E. and C0 groups (p>0.05).



GRAPH 1: Changes in serum Vit C (mg/dL) of DIE, DE, C.E. and C0 groups.

N.B.: values are graphed in bars showing vit C values in their means. p<0.01 as highly significant (**), p<0.05 as significant (*) and p>0.05 as not significant (ns). Group names DIE, DE, C.E. and C0 are explained in Table-1.

GRAPH-1 shows serum vitamin C values having a significant increase in the DIE group (p>0.05), D.E. group (p<0.05) and C.E. group (p<0.01), whereas non-significant change in the C0 group (p>0.05).



GRAPH 2: Changes in serum MDA (nm/mL) of DIE, DE, C.E. and C0 groups.

N.B.: values are graphed in bars showing vit C values in their means. p<0.01 as highly significant (**), p<0.05 as significant (*) and p>0.05 as not significant (ns). Group names DIE, DE, C.E. and C0 are explained in Table-1.

GRAPH-2 shows serum MDA values significantly decreased in the DIE group (p<0.01), D.E. group (p<0.05) and C.E. group (p<0.05), but insignificant changes in the C0 group (p>0.05).

DISCUSSION

There was no significant difference in the FBS, PPBS and HbA1c before the supplementation. But after six months of supplementation of vitamin E, FBS level in the DIE ($p<0.001$), the D.E. ($p<0.01$) group significantly decreased. But in C.E. and C0 groups, significant changes were found in the FBS level. Similarly, PPBS level (mg/dL) in DIE ($p<0.001$) & D.E. ($p<0.01$) level significantly decreased supplementation of vit-E as compared to their previous value. But in C.E. and C0 groups, insignificant ($p>0.05$) changes were found.

The HbAc% was found to decrease significantly in the DIE group, but in other groups (D.E., C.E., C0), there were insignificant changes ($p>0.05$) found after the vit E supplementation. These changes in FBS & PPBS values in diabetic and healthy rabbits can be explained by the ability of vit E to reduce hyperglycemia. This may be brought by the suppression of glucose output and gluconeogenesis from the liver, which was linked to lipolysis prevention in adipose tissue. According to Shamsi MSA et al. research, vit E treatment in diabetic rats led to decreasing circulating levels of glucagon. It is essential to preserve glucose homeostasis as it plays a vital part in regulating hepatic metabolic activities and maintaining blood glucose concentration within the normal range. [27, 28]. Jain N et al. study on diabetic rabbits suggested that vit E prevents hyperglycemia by scavenging the reactive oxygen species (ROS). [29].

In Diabetes, undesirable more circulating glucose is a reactant molecule involved in the glycosylation of other tissues and biomolecules. Glycosylation of haemoglobin is of considerable value in clinical utility and is very popular. Glycosylated haemoglobin (HbA1c) is formulated as a percentage (%) of total haemoglobin concentration, and it gives a distinct past evaluation of the mean plasma glucose concentration during the last 6-8 weeks; its evaluation is believed as a very reliable method of evaluation of glycemic control. The higher the HbA1c % in the diabetic animal, the lower the mean diabetic control. [30 - 32]. It is surmised that glycemic control will reduce the long-term complications of Diabetes mellitus. Once a haemoglobin molecule has been glycated, it stays inside the red blood cell till its life span (120 days). Many studies have shown that HbA1c is an index of average blood glucose over the preceding few weeks and months. The HbA1c does not reflect the exact mean blood glucose; somewhat, it is weighted relatively towards recent levels [33]. The mean glycemia during the month preceding the measurement of HbA1c contributes 50% of the result, and 30-60 days before the measurement contributes the final 25% [34].

In T2DM cases, Vit-E intake caused improved glycaemic control in many studies. [35 – 36] Supplemented vit E prohibits Hb from glycation; it interrupts glycosylation of Hb in the Maillard reaction at an early step. It also inhibits Advanced Glycation End product (AGE) formation. [36 -37]. It preserves pancreatic β- cell function and reverses β-cell apoptosis. [38 – 39]. Some studies also revealed that the supplementation of

vit E in T2DM cases having low serum vit E concentration caused a very noticeable effect on serum HbA1c levels and fasting insulin levels. [40 - 41]

In a study on STZ-induced Diabetes in male albino rats, supplementation of Vit E for 16 weeks revealed the reversal of most biochemical parameters, including a significant decrease in FBS, HbA1c and lipid profile levels [42]. In another study on Wistar rats (normal and STZ-induced diabetic), 400-500 IU/Kg vit E supplementation for 8 months recorded a significant decrease ($p<0.001$) in their FBS, HbA1c levels in vit E supplemented group as compared to the control group. [43].

Numerous plasma compounds in different concentrations exhibit antioxidant properties; vit E, vit C, and reduced glutathione are some of the plasma compounds acting as antioxidants by scavenging the free radicals and acting on the propagation phase of lipid peroxidation [44]. Any variations in their levels are significant O.S. markers. But in certain conditions, these vitamins also promote toxicity by production of pro-oxidants. Vit E levels in different studies showed different concentrations in diabetic cases [45]. The harmful effects on vascular changes in diabetic animals and humans were reported by many reviewers [46]. Like other studies, our study revealed markedly reduced vitamin C levels in diabetic animals [47 – 50].

The increase in vitamin C status after supplementation with vit E is more difficult to explain in GRAPH-1. Still, it might be linked to increased absorption and/or decreased plasma clearance of ascorbic acid. [51 - 52]. D.M. produces disturbances in the body's lipid profile which causes cell susceptibility to lipid peroxidation. [53]. Due to multiple bonds, polyunsaturated fatty acids of the cell membranes are prone to attack by F.R., as reported in many experimental studies. [54]. Through intermediate reactions of F.R., lipid hyper peroxidation (LHP) generates more lipid radicals that are so toxic and reactive that it forms new LHP. As a result of lipid peroxidation Malondialdehyde (MDA) is formed, which is used to measure lipid peroxidation by Thiobarbituric acid (TBA) reaction. [44, 55 -57].

Figure-2 shows a significant decrease in MDA level ($p<0.01$) in all vitamin E-supplemented groups compared to their basal values. Similar results were also found in other studies. An author demonstrated that supplementation of streptozotocin (STZ) diabetic rats with vitamin C, E and beta-carotene for 8 weeks significantly reduced MDA and GSH (glutathione) levels. [58]. In another study, treatment with vit E & C significantly lowered MDA and GSH levels compared to diabetic animals without any supplementation. [59]. Another study in the STZ-diabetic rat aorta demonstrated that supplementation with vitamin E significantly lowers the lung and liver MDA concentrations and causes improvement in impaired endothelium-dependent vasorelaxation [60].

SUMMARY & CONCLUSIONS

In Vit E pre-clinical study, it was found ineffective in maintaining weight in diabetic rabbits. Data suggests vit E to be beneficial in maintaining fasting, postprandial blood sugar, and diminishing HbA1c% in experimental animals. Similarly, vit E is beneficial in decreasing O.S. marker MDA and increasing A.O.

status, as shown by increasing levels of vit C level. Supplementation of vit E significantly reduces oxidative stress induced by D.M. (as indicated by reduction in MDA levels and increase in vit C levels), but also had reduced effect on the hyperglycaemic state. Our findings support the A.O. supplementation like vit E in preventing DM-induced OS and suggest potential therapeutic benefits for reducing diabetic conditions and their associated complications.

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REFERENCES

1. Ogunmodede OS, Saalu LC, Ogunlade B, Akunna GG, Oyewopo AO. An evaluation of the hypoglycemic, antioxidant and hepatoprotective potentials of Onion (Allium Cepa L.) on Alloxan-induced Diabetic Rabbits. Int J Pharma 8(1): 21-9, 2012. ISSN 1811-7775/ DOI: 10.3923/ijp.2012.21.29.
2. Ogugua VN. Assessment of some parameters of oxidative stress in alloxan-induced diabetic rabbits. PhD Thesis, Dept of Biochemistry, University of Nigeria, Nsukka, 2000: 205.
3. Galluzzo A, Giordano C, Bompiana GD. Cell-mediated immunity in diabetic pregnancy. Immunology of normal and diabetic pregnancy. John Wiley & Sons New York, 1990: 273-8.
4. Aruoma OI, Kaur H, Halliwell B. Oxygen free radicals and human disease. Journal of Royal Society Health, 1991; 8: 172-7.
5. Krishna MI, Das UN. Prevention of chemically induced diabetic mellitus in experimental animals by polyunsaturated fatty acids. Nutrition, 2001; 17 (2): 126-51.
6. Ogugua VN, Ikejiaku CA. Effects of Palm oil on some oxidative indices of Alloxan-induced diabetic rabbits. Animal Research International, 2005; 2 (1): 227-30.
7. Rondall LD, Christy LL, Melissa AA, Patrick MM, Thomas ET. Differential indicators of diabetes-induced oxidative stress in New Zealand white rabbits: Role of dietary vitamin E supplementation. Int. Jnl. Experimental Diab. Res., 2002; 3: 185-92. DOI: 10.1080/15604280290013892.
8. Rosen P, Du X, Tschope D. Role of oxygen-derived radicals for vascular dysfunction in the diabetic heart: Prevention by alpha-tocopherol? Mol. Cell Biochem, 1998; 188: 103-11.
9. Vannucchi H, Araujo WF, Bernardes MM, Jordao AA. Effect of different vitamin E levels on lipid peroxidation in streptozotocin-diabetic rats. Int. J. Vitam. Nutr. Res., 1999; 69: 250-4.

10. Rosen P, Du X, Tschope D. Role of oxygen-derived radicals for vascular dysfunction in the diabetic heart: Prevention by alpha-tocopherol? Mol Cell Biochem, 1998; 188:103-11.
11. Vannucchi H, Araujo WF, Bernardes MM, Jordao AA. Effect of different vitamin E levels on lipid peroxidation in streptozotocin-diabetic rats. Int. J. Vitam. Nutr. Res., 1999; 69: 250-4.
12. Muma AF. Lipid peroxidation in malaria patients. MSc Dissertations, Dept of Biochemistry, University of Nigeria, Nsukka, 1994: 145.
13. Guthridge JMC. Reactivity of hydroxyl and hydroxyl-like radicals discriminated by the release of TBARS from deoxyribose, dudeosides and Beuroate. Biochemistry, 1994; 224: 761-7.
14. Takemoto K, Doi W, Masuoka N. Protective effect of vitamin E against alloxan-induced mouse hyperglycemia. Biochimica et Biophysica Acta, 2016; 1862: 647-50. http://dx.doi.org/10.1016/j.bbadis.2015.12.022.
15. Masukawa T, Nakanishi K. Involvement of blood glucose in the dimethyl thiourea-induced protection against alloxan-induced Diabetes. Jpn. J. Pharamacol, 1994; 66: 159-61.
16. Hamden K, Carreau S, Jamoussi K, Miladi S, Lajmi S, Aloulou D, Ayadi F, Elfeki A. 1α, 25-dihydroxy vitamin D3: therapeutic and preventive effects against oxidative stress, hepatic, pancreatic and renal injury in alloxan-induced Diabetes in rats. J. Nutr. Sci. Vitaminol, 2000; 55: 215-22.
17. Yaghmasi P, Esfahani-Najad H, Ahmad R, Hayati-Roodbari N, Ebrahim-Habibi A. Material zinc intake of Wister rats has a protective effect in the alloxan-induced diabetic offspring. J. Physiol. Biochem., 2013; 69: 35-43.
18. Slonim AE, Surber ML, Page DL, Sharp RA, Burr IM. Medication of chemically induced Diabetes in rats by vitamin E. J. Clin. Invest. 1983; 71: 1282-8.
19. Olanlokun JO. The protective influence of vitamin E on the antioxidant defence system of the whole blood and liver of formal and alloxan-induced diabetic rats. Indian J. Clin. Biochem, 2008; 23: 62-6.
20. Kamimura W, Doi W, Takemoto K, Ishihara K, Wang DH, Sugiyama H, Oda S, Masuoka N. Effect of vitamin E on alloxan-induced mouse diabetes. Clin Biochem, 2013; 46: 795-8.
21. Mayer-Davis EJ, Bell RA, Reboussin BA, Rushing J, Marshall JA, Hamman RF. Antioxidant nutrient intake and diabetic retinopathy: The Son Luis Valley diabetes study. Ophthalmology, 1998; 105: 2264-70.
22. Gazis A, White DJ, Page SR, Cockcroft JR. Effect of oral vitamin E (alpha-tocopherol) supplementation on vascular endothelial function in type 2 diabetes mellitus. Diabetes Med., 1999; 16: 304-11.
23. Saadia SA, Khan AH, Sirhindi GA, Khan SU. Alloxan-induced Diabetes in rabbits. Pak J. Pharmacol., 2005; 22(2): 41-5.
24. Nimbalkar VV, Mandlik RV, Naik SR, Masseh A. Nonenzymatic glycosylation: A biochemical link between chronic hyperglycemia and pathophysiologic processes

associated with diabetic complications and ageing-related debilities. Biomed Age Patho., 2012; 2(4): 133-42.

25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem., 1979; 95: 351-8.
26. George J. Ascorbic acid concentrations in dimethylnitrosamine-induced hepatic fibrosis in rats. Clinica Chimica Acta, 2003; 335: 39-47.
27. Postic C, Dentin R, Girard J. Role of the liver in the control of carbohydrate and lipid homeostasis. Diabetes Metab, 2004; 30: 398-408.
28. Shamsi MSA, Amin A, Adeghate E. Beneficial effect of vit E on the metabolic parameters of diabetic rats. Molecule Cellul Biochem, 2004; 261 (1): 35-42.
29. Jain N, Naseem I, Ahmad J. Evaluation of DNA damage and metabolic syndrome parameters in diabetic rabbits supplemented with antioxidants. Funda Clin Pharmacol, 2009; 23 (2): 197-205.
30. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical management of hyperglycemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association for the study of Diabetes. Diabetologia, 2009; 52 (1): 17-30.
31. Dandson MB, Schriger DL. Relationship between fasting plasma glucose and glycosylated haemoglobin. J Ame Med Asso, 1999; 281 (13): 1203-10.
32. Akinloye OA, Adaramoye OA, Akinlade KS, Odetola AA, Raji AA. Relationship between fasting plasma glucose and glycosylated haemoglobin in adult diabetic Nigerians, Afri J Biomed research, 2007; 10: 127-32.
33. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care, 1995; 18: 440-7.
34. Beach K.W. A theoretical model to predict the behaviour of glycosylated haemoglobin levels. J Theor Biol, 1979; 81: 547-61.
35. Farvid MS, Jalali M, Siassi F, Saadat N, Hosseini M. The impact of vitamins and/or mineral supplementation on blood pressure in type 2 diabetes. J Am Cell Nutr, 2004; 23 (3): 272-9.
36. Ceriello A, Giugliano D, Quatraro A, et al. Vitamin E reduction of protein glycosylation in Diabetes, New prospect for prevention of diabetic complication? Diabetes Care, 1991; 14: 68-72.
37. Minamiyama Y, Takemura S, Bito Y, Shinkawa H, Tsukioka T, et al. supplementation of alpha-tocopherol improves cardiovascular risk factors via the insulin signalling pathway and reduction of mitochondrial reactive oxygen species in type II diabetic rats. Free Radic Res, 2008; 42: 261-71.
38. Jin L, Xue HY, Jin LJ, Li SY, Xu YP. Antioxidant and pancreas-protective effect of aueubin on rats with streptozotocin-induced Diabetes. Eur J Pharmacol, 2008; 582: 162-7.
39. Pazdro R, Burgess JR. The role of vitamin E and oxidative stress in diabetes complications. Mech Ageing Dev, 2010; 131: 276-86.

40. Suksomboon N, Poolsup N, Sinprasert S. Effects of vitamin E supplementation on glycaemic control in type 2 diabetes: systematic review of randomized controlled trials. J Clin Pharm Ther, 2011; 36 (1): 53-63.
41. Xu R, Zhang S, Tao A, Chen G, Zhang M. Influence of vitamin E supplementation on glycaemic control: A meta-analysis of Randomized controlled trials. PLoS One, 2014; 9 (4): e95008.
42. Halim EM, Mukhopadhyay AK. Effect of Ocimum Sanctum (Tulsi) and vitamin E on biochemical parameters and retinopathy in streptozotocin-induced diabetic rats. Indian Journal of Clinical Biochemistry, 2006; 21 (2): 181-8.
43. Yulek F, Meral O, Cndan O, Isik AC, Nurar A, Stefek M, Bauer V, Karasu C. Effects of Stobadine and vitamin E in diabetes-induced retinal abnormalities: Involvement of oxidative stress. Arch Medi Research, 2007; 38: 503-11.
44. Bonnefont RD, Bastard JF, Jaudon MC, Delattree J. Consequences of the diabetic status on the oxidant/ antioxidant balance. Diabetes Metab, 2000; 26 (3): 163-76.
45. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress- A concise review. Saudi Pharmaceutical Journal, 2015.
46. Maritim AC, Sanders RA, Watkins III JE. Effects of α-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. Journal of Nutritional Biochemistry, 2003; 14: 288-94.
47. Wilt JC, Byer T. Does diabetes mellitus increase the requirement for vitamin C? Nutr Rev, 1996; 54: 193-202.
48. Garg MC, Singh KP, Bansal DD. Effect of vitamin E supplementation on antioxidant status of diabetic rate. Med Sci Res, 1996; 24: 325-6.
49. Kumari K, Sahib MK. Susceptibility of different rat tissues to non-enzymatic protein glycosylation. Indian J Exp Biol, 1993; 31: 194.
50. Niki E. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. Am J Clin Nutr, 1991; 54: 95-111.
51. Stayanovsky DA, Goldman R, Darrow RM, Organisciak DT, Kagan VE. Endogenous ascorbic acid regenerates vitamin E in the retina directly and in combination with exogenous dihydrolipoic acid. Current Eye Research, 1995; 14: 181-9.
52. Halpner AD, Hanndelman GJ, Belmont CA, Harris JM, Blumberg JB. Protection by vitamin C of oxidant-induced loss of vitamin E in rat hepatocytes. J Nutri Biochem, 1998; 9: 355-9.
53. Patricia PM. Reactive species and Diabetes counteracting oxidative stress to improve health. Curr Opin Pharmacol, 2009; 9: 771-9.
54. Butterfield DA, Koppal T, Beverly H, Ram S, Nathan H, Kenneth H, et al. Structural and functional changes in proteins induced by the antioxidants N-tert-butyl-alpha-phenylnitrone and vitamin E, Ann N Y Acad Sci, 1998; 854: 448-62.
55. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J, 2012; 12 (1): 5-18.

56. Esterbauer H, Schaur RJR, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal malonaldehyde and related aldehydes. Free Rad Biol Med, 1991; 11 (1): 81-128.
57. Hatice P, Banu S, Neslihan B. Lipid peroxidation and resistance to oxidation in patients with type 2 diabetes mellitus. Tohuku J Exp Med, 2004; 203: 211-8.
58. Mekinova D, Chorvathova V, Volkovova K, Staruchova M, Grancicova E, Klvanoca J, Ondreicka R. Effect of intake of exogenous vitamins C, E and beta-carotene on the antioxidative status in kidneys of rats with streptozotocin-induced diabetes. Nahrung, 1995; 39 (4): 257-61.
59. Kedziora KK, Szram S, Kornatoueski T, Szadujkis SL, Kedziora J, Bartosz G. Effect of vitamin E and vitamin C supplementation of antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. Exp Nephrology, 2003; 95: 134-43.
60. Cinar M, Ulker S, Alper G, Evinc A. Effect of dietary vitamin E supplementation on vascular reactivity of thoracic aorta in streptozotocin-diabetic rats. Pharmacology, 2001, 61 (1): 56-64.