The Effect of Antioxidantson Electrolytes in Vancomycin-Streptozotocin InducedDiabetes Kidney Disease inRabbits.

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Abstract

Diabetes mellitus is a metabolic disease that is characterized by relative or absolute insulin deficiency, diabetic kidney disease (DKD) seems to be one of the most common complications of diabetes mellitus. Diabetic kidney disease was known as diabetic nephropathy (DN) and is the single strongest predictor of mortality in patients with diabetes. Forty male and female rabbits weighing 1000-1300 mg were divided randomly into five groups. Diabetes mellitus was induced in the overnight fasted rabbits by a single IP injection ofStreptozotocin in dose of 50 mg/kg. Then animals were started of antioxidant treatment, blood sampling were taken each 2 weak, the laboratory analysis which includes blood sugar, and serum electrolytes (potassium, sodium, and chloride).The various antioxidants were used in different combination in treated 1 group (quercetin15 mg/kg and L-carnitine15 mg/kg) and treated 2 group (quercetin15 mg/kg, L-carnitine15 mg/kg, Thioctic acid 20mg/kg, Vitamin C 15mg/kg) orally.There was a non-significant decrease in serum glucose level in treated 1 group andtreated 2 group (which treated with different combination of antioxidants) compared with diabetic control group and significant increase in serum (potassium, and chloride) but non-significant increase in serum sodium in treated groups compared with diabetic control group.The role of antioxidants as adjuvant therapy to decrease and prevent diabetic kidney disease through the scavenging effect to reactive oxygen species produced by diabetic kidney tissues.

Key words: *Antioxidant; Diabetes mellitus; DKD;quercetin; Thioctic acid;L-carnitine; Vitamin C.*

Introduction

Diabetes mellitus (DM) is one of the main threats to human health in the 21st century because changes in humanbehavior and lifestyle during the last century have resulted in an increase in the incidence of diabetesworldwide.[1]Diabetic nephropathy (DN) is a common microvascular complication in type 1 and type 2 diabetes mellitus.[2]

Diabetic nephropathy is a cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD). It is a progressive and irreversible kidney disease that is characterized by initial hyperfiltration, albuminuria, expansion of mesangial matrix, interstitial fibrosis, thickening of basement membranes, and renal cell damage. DN affects on 20–30% of the diabetic patients.[3]

Oxidative stress in patients with CKD appears due to increase oxidant activity and decrease antioxidant system.[4]The pathogenesis of DN is not yet fully understood, however, several genetic and environmental factors that can impaired health-related quality of life of patients. Hyperglycemia in DM leads to the development of an array of metabolic, biochemical and hemodynamic alteration in renal tissues.[5]

There are many antioxidant agents were used for delaying diabetic kidney disease progression and restore the antioxidant defense system thereby preventing ROS mediated injuries like quercetin, L-carnitine, Thioctic

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acid, and Vitamin C.Many studies show that antioxidants alleviate renal injury and improve kidney function via reducing oxidative damage and/or inflammation, the reno-protective effects of ascorbate may derive from its known antioxidant activity in scavenging source and derived ROS, including non-radical oxidants.[6]

Materials and Methods

Animal care

Forty male and female rabbits weighing 1000-1300 mg. They were obtained from a local market in Basrah kept separated to five groups (eight rabbits in each group) at the animal house of College of Pharmacy / University of Basrah for 2 weeks for acclimatization before starting the experiment. The rabbits were housed in a light controlled, an air –conditioned atmosphere at a constant temperature and supplied with food *ad libitum*. The rabbits were fed a trefoil, bread and lettuce with a free access to tap water. Noisiness and tough handling were avoided to reduce stress.The animals used in this study were treated in conformity to the National Institute of Health (NIH) guidelines for handling laboratory animals.

Preparation of antioxidant supplementation

The antioxidantsupplementationwas bought from private pharmacies in Basrah as tablets. The tablets were crushed by using a mortar to convert them to powder,then dissolved in distilled water to obtain suitable form of solution or suspension for administration orally to the rabbits. The various antioxidants were used in different combination treated 1 group (quercetin15 mg/kg and L-carnitine15 mg/kg) and treated 2 group (quercetin15 mg/kg, L-carnitine15 mg/kg, Thioctic acid 20mg/kg, and Vitamin C 15mg/kg) orally.

Preparation of vancomycin

Vancomycin was dissolved in normal saline to obtain a solution concentration (200mg/ml). A dose of (200mg /kg) of body weight was injected intra peritoneum (IP) for each rabbit in specific groups (Diabetic Control group, treated 1 group, treated 2 group, and Vancomycin control group.

Induction of diabetes mellitus

Diabetes mellitus was induced in the overnight fasted rabbits by a single IP injection ofStreptozotocin in dose 50mg/kg of body weight. Streptozotocin was dissolved in citrate buffer solution (pH 4.5) and freshly prepared before injection. After 5 hour of injection, 5% glucose water was given instead of drinking water to overcome the high insulin released to all rabbits injected with Streptozotocin that cause hypoglycemia. Hyperglycemia in rabbits was followed up within 5 days byear vein tipping using glucometer Accu-Chek active meter in animal house, farther check up by blood sampling and analyze it. Rabbits with random blood sugar concentration more than 200 mg/dl were considered as diabetic.

Treatment

After induction of diabetes mellitus vancomycin was given to increase the renal injury of diabetic rabbits by two doses of vancomycin, then the animals were started with antioxidant treatment, blood sampling were taken each 2 weak 4 times during the study.The treatment with antioxidant agents lasted for a period of 40 days, the animals grouping, drugs dosing, and duration of treatments were shown in Table (1).

Table 1. Summary of animals grouping, drugs dosing, and duration of treatments.

Group	Treatment	Duration of treatment
Group 1 (Normal Control)	-citrate buffer solution 1ml/kg IP -normal saline 1ml/kg IP -Distilled water (4ml/kg/day) orally	-once only -twice only -40 days
Group 2 (Diabetic Control)	-streptozotocin buffer solution1ml/kg(50mg/kg) IP -vancomycin -solution 1ml/kg (200mg/kg) IP Distilled water (4ml/kg/day) orally	-once only -twice only -40 days
Group 3 (Treated 1)	-streptozotocin buffer solution1ml/kg(50mg/kg) IP -vancomycin solution1ml/kg(200mg/kg) IP - Solution of two antioxidant quercetin ml/kg (15 mg/kg) and L-carnitine1ml/kg (15 mg/kg), (4 ml/kg/day of total solution of antioxidants with D.W. orally	-once only -twice only -40 days
Group 4 (Treated 2)	-streptozotocin buffer solution 1ml/kg (50mg/kg) IP -vancomycin solution1ml/kg(200mg/kg) IP -Solution of four antioxidant quercetin ml/kg (15 mg/kg) + L-carnitine1ml/kg (15 mg/kg) + Thioctic acid 1 ml/kg (20mg/kg) + Vitamin C 1ml/kg (15mg/kg), (4 ml/kg/day of total solution of antioxidants with D.W. orally	-once only -twice only -40 days
Group 5 (Vancomycin control)	-citrate buffer solution1ml/kg IP -vancomycin solution 1ml/kg (200mg/kg) IP -Distilled water (4ml/kg/day) orally	-once only -twice only -40 days

Assays

The following analysis were done: blood sugar, serum potassium, serum sodium, serum chloride, by specific kit for each one. The kit were supplied from COBAS -Roche (Germany) Supplier.

Statistical Analysis

Data are expressed as Means ± standard deviation (SD) of samples. Analysis was made by using SPSS (statistical package for social sciences) for Windows (version 23) and Microsoft office (2016).Differences among different groups were compared by one-way analysis of variance (ANOVA). Statistically significant was accepted at a level of$P \leq 0.05$.[7]

Results

Random blood sugar

As shown in Table 2 a significant ($P<0.001$) increase in random blood sugar for three Diabetic induced groups (diabetic control, treated one, andtreated two) compared to the normal control group and Vancomycin control group in each blood sample. A non-significant difference ($P>0.05$) of treated one and treated two groups compared with Diabetic control group in each blood sample. A non-significant difference between

Vancomycin control group and normal control group (P>0.05) for each blood sample.

Table 2. Effect of different antioxidants and vancomycin on serum glucose level.

Group	Serum glucose (mg/dL)			
	Blood sample 1	**Blood sample 2**	**Blood sample 3**	**Blood sample 4**
Normal Control	101.62 ± 8.68	104.93 ± 6.96	106.48 ± 13.81	113.20 ± 11.05
Diabetic Control	236.50 ± 26.14 ***a ***c	233.72 ± 25.36 ***a ***c	200.09 ± 14.89 ***a ***c	180.85 ± 17.10 ***a ***c
Treated one	232.00 ± 23.74 ***a ***c	221.26 ± 22.40 ***a ***c	185.63 ± 24.67 ***a ***c	173.11 ± 23.21 ***a ***c
Treated two	231.62 ± 24.62 ***a ***c	201.29 ± 18.16 ***a ***c	189.18 ± 26.33 ***a ***c	181.88 ± 12.70 ***a ***c
Vancomycin control	103.75± 14.00	102.54 ± 7.45	112.33 ± 17.53	112.31 ± 16.96

Values expressed as mean + standard error. N=8 for each group.

* = significant at p<0.05; ** = significant at p<0.005; *** significant at p<0.001

a= significant difference when compared with normal control group at the same period; b= significant difference when compared with diabetic control group; c= significant difference when compared with vancomycin control group.

Serum Potassium

As shown in Table3 anon-significant difference (P>0.05) between groups in blood sampling 1, then become significant decrease of serum potassium in diabetic control group (P< 0.001) and vancomycin control group (P=0.001) compared to normal control groupin blood sampling 2.Moreover,significant decrease of serum potassium in diabetic controlgroup (P=0.002) and vancomycin control group (P=0.037) compared to normal control group in blood sampling 3, then become non-significant difference (P>0.05) between groups in blood sampling 4.A significant difference of treated one group (P=0.002) and treated two group (P=0.003) compared with diabetic control group in blood sampling 2.A significant difference of treated one group (P=0.001) and treated two group (P=0.041) compared with diabetic control group in blood sampling 3, and non-significant difference (P>0.05) of treated one group and treated two group, compared with diabetic control group in blood sampling 4.

Table 3. Effect of different antioxidants and vancomycin on serum potassium level.

Group	Serum Potassium (mmol/L)			
	Blood sample 1	Blood sample 2	Blood sample 3	Blood sample 4
Normal Control	5.18 ± 2.03	4.31 ± 0.60	4.20 ± 0.58	3.88 ± 0.56
Diabetic Control	4.02 ± 0.51	2.86 ± 0.56 ***a	3.17 ± 0.64 **a	3.61± 0.87
Treated one	4.00 ± 0.40	3.81± 0.42 **b	4.25 ± 0.47 **b	3.41± 0.58
Treated two	4.1 ± 0.50	3.77± 0.60 **b	3.83 ± 0.67 *b	3.66 ± 0.93
Vancomycin control	4.57 ± 2.05	3.33 ± 0.57 **a	3.52 ± 0.72 *a	3.61 ± 0.48

Values expressed as mean + standard error. N=8 for each group.

* = significant at p<0.05; ** = significant at p<0.005; *** significant at p<0.001

a= significant difference when compared with normal control group at the same period; b= significant difference when compared with diabetic control group; c= significant difference when compared with vancomycin control group.

Serum Sodium

As shown in Table 4a significant decrease of serum sodium in diabetic control group (P=0.021), treated one group (P=0.026) and treated two group (P=0.038) compared with normal control groupin blood sampling 1. A significant difference (P=0.006) of diabetic control group compared with normal control group in blood sampling 2.A significant difference (P=0.009) of diabetic control group compared with normal control group in blood sampling 3.In addition, significant difference (P=0.011) of diabetic control group compared with normal control group in blood sampling 4.

A non-significant difference (P>0.05) of treated one group and treated two group compared with diabetic control group in each blood sampling.A significant difference (P=0.014) of vancomycin control group compared with normal control group in blood sampling 2 (after starting of vancomycin).A significant difference (P<0.001) of vancomycin control group compared with normal control group in blood sampling 3. In addition, significant difference (P<0.007) of vancomycin control group compared with normal control group in blood sampling 4.

Table4. Effect of different antioxidants and vancomycin on serumsodium level.

Group	Serum Sodium (mmol/L)			
	Blood sample1	Blood sample2	Blood sample3	Blood sample4
Normal Control	142.00 ± 3.20	140.00 ± 2.87	140.37 ± 3.33	141.00 ± 3.02
Diabetic Control	138.37± 2.44 *a *c	124.50 ± 9.25 *a	130.50 ± 11.18 *a	131.50 ± 5.34 *a
Treated one	138.50 ±2.32 *a *c	130.87 ± 10.70	135.87 ± 4.51 **c	136.50 ± 7.54
Treated two	138.75 ± 3.69 *a *c	128.50 ± 14.17 *a	135.25 ± 6.86 *c	134.25 ± 9.17
Vancomycin control	141.62 ± 3.15	126.25 ± 12.20 *a	125.62 ± 7.28 ***a	130.75 ± 8.59 **a

Values expressed as mean + standard error. N=8 for each group.

* = significant at p<0.05; ** = significant at p<0.005; *** significant at p<0.001

a= significant difference when compared with normal control group at the same period; b= significant difference when compared with diabetic control group; c= significant difference when compared with vancomycin control group.

Serum Chloride

As shown in Table 5 anon-significant difference (P>0.05) between groups in blood sampling 1. Then become significant decrease of serumchloride in diabetic control group (P< 0.001) and vancomycin control group (P=0.036) compared with normal control group in blood sampling 2 (after starting of antioxidants and vancomycin).A significant difference (P=0.039) of diabetic control group and vancomycin control group (Pt=0.04) compared to normal control group in blood sampling 3.A significant difference of diabetic control group (P= 0.03) and vancomycin control group (P=0.037) compared to normal control group in blood sampling 4.

Asignificant difference of treated one group (P=0.05) and treated two group (P= 0.039) compared with diabetic control group in blood sampling 2. Asignificant difference of treated one group (P=0.018) and treated two (P= 0.05) group compared with diabetic control group in blood sampling 3.A significant difference of treated one group (P=0.016) and treated two (Pt= 0.01) group compared with diabetic control group in blood sampling 4.

Table 5. Effect of different antioxidants and vancomycin on serumchloride level.

Group	Serum Chloride (mmol/L)			
	Blood sample1	Blood sample2	Blood sample3	Blood sample4
Normal Control	103.50 ± 3.29	103.50 ± 4.44	102.12 ±3.56	101.00 ± 3.07
Diabetic Control	101.00 ± 2.61	84.50 ± 11.27***a	86.12 ± 12.14 *a	89.75 ± 12.04 *a
Treated one	99.75 ± 1.66	94.00 ± 10.91 *b	97.00 ± 7.89 *b	95.75 ± 14.67 *b
Treated two	99.75 ± 2.60	94.87 ± 11.128 *b	94.87 ± 9.07 *b	93.37±10.41 *b
Vancomycin control	102.00 ± 2.92	93.00 ± 8.73 *a	86.87 ± 8.83 **a	91.25±11.32 *a

Values expressed as mean + standard error. N=8 for each group.

* = significant at p<0.05; ** = significant at p<0.005; *** significant at p<0.001

a= significant difference when compared with normal control group at the same period; b= significant difference when compared with diabetic control group; c= significant difference when compared with vancomycin control group.

Discussion

The local and systemic oxidative stress that underlies the pathological features ofdiabetic nephropathy is the result of the imbalance in the production of oxidants/ antioxidants,since the powerful antioxidant mechanisms is balanced the oxidative aggression. Some antioxidants have been demonstrated to modulate multiple cell signaling molecules such as the pro inflammatory cytokines, transcription factors, apoptosis proteins, and various endogenous antioxidants.[8]

Diabetic control group showed increase level of serum glucose and decrease levels of serum (potassium, sodium, and chloride). There was a non-significant decrease in serum glucose level in treated groups compared with diabetic control group. The effect of antioxidants in decreasing serum glucose level may be depending on the dose and duration of antioxidants treatment. Some studies showed the role of antioxidants like quercetin that has a positively influences on glucose metabolism in the skeletal muscle and liver, the quercetin is from flavonoid family hasthe most potent anti-oxidant activity.[9]

There was a significant increase in serum (potassium, and chloride) and non-significant increase in serum sodium in treated groups compared with diabetic control group.Some studies show the role of antioxidants in improvement the serum potassium level in diabetic kidney disease like use combination of vitamins A, vitamin C, α-lipoic acid and other antioxidant supplement that increase of serum potassium level in diabetic treated group.[7]

Other study shows the effect of sweet potato extract indecrease of serum sodium level because of its constituents of antioxidants like flavonoids, tannins and others.[10]The effect of antioxidant supplement in diabetic kidney dysfunction is decrease oxidative stress and restore the electrolyte levels that include serum chloride and another electrolyte.[11]

Vancomycin control group showed a significant decrease in serum(potassium, chloride, and sodium). Karimzadeh et al. show development of hypokalemia during 2 to 3 days of initiating of vancomycin.[12]

Conclusion

The study examined the effect of antioxidant agents and kidney function status in streptozotocin induced diabetic rabbits, there was a significant increase in serum (potassium, and chloride) and non-significant increase in serum sodium in treated groups compared with diabetic control group. The results therefore suggest that the antioxidant agents may be useful in ameliorating the effect of diabetes and oxidative stress related kidney dysfunction and the use of antioxidants as adjuvant therapy in decrease and prevent diabetic kidney disease.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Consideration: Ethical permission was taken from ethics review committee of college of pharmacy/ University of Basrah.

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References

1. I. Khushk, M. U. Dahot, S. A. Baloach, and M. A. Bhutto, "The Evaluation of Soybean Extracts in Alloxan-Induced Diabetic Rabbits", Biotechnology and genetic engineering,2010, 8: 22-25.
2. J. F. Ndisang and A. Jadhav, "Hemin therapy improves kidney function in male streptozotocin-induced diabetic rats: Role of the heme oxygenase/ atrial natriuretic peptide/adiponectin axis", Endocrinology, 2014, 155(1): 215-229.doi: 10.1210/en.2013-1050.
3. N. Al-Waili, H. Al-Waili, T. Al-Waili, and K. Salom, "Natural antioxidants in the treatment and prevention of diabetic nephropathy; a potential approach that

warrants clinical trials," Redox Rep., 2017, 22(3): 99-118.doi: 10.1080/13510002.2017.1297885.

4. A. Y. Putri and M. Thaha, "Role of oxidative stress on chronic kidney disease progression",Acta Med. Indones., 2014, 46(3): 244-252.
5. A. D. Kandhare, A. Mukherjee, and S. L. Bodhankar, "Antioxidant for treatment of diabetic nephropathy: A systematic review and meta-analysis", Chem. Biol. Interact., 2017, 278: 212-221.doi: 10.1016/j.cbi.2017.10.031.
6. J. M. Dennis and P. K. Witting, "Protective role for antioxidants in acute kidney disease". Nutrients, 2017,9(7), 718:1-25.doi: 10.3390/nu9070718.
7. N. C. Chinaka, L. C. Chuku, C. C. Monago-Ighorodje, and O Edeogu, "Antioxidant Supplementation and Kidney Function Status of Wistar Rats Following High Fat Diet-Streptozotocin (Hft-Stz) Induced Type 2 Diabetes", Biochem Mol Biol J., 2018, 4(3), 24:1-6. doi: 10.21767/2471-8084.100073
8. A. G. Miranda-díaz, L.Pazarín-villaseñor, F. G.Yanowsky-escatell, J. Andrade-sierra, and E. K. Changes, "Oxidative Stress in Diabetic Nephropathy with Early Chronic Kidney Disease", J. Diabetes Res., 2016, 2016: 1-7.
9. H. M. Eid, A.Nachar, F. Thong, G.Sweeney, and P. S. Haddad,"The molecular basis of the antidiabetic action of quercetin in cultured skeletal muscle cells and hepatocytes",Pharmacogn Mag., 2015, 11(41): 74-81.
10. O.O. Ogunrinola, O.O. Fajana, S.N. Olaitan, O.B. Adu and M.O. Akinola, "Anti-Diabetic Activity of Ipomoea batatas Leaves Extract: Effects on Hepatic Enzymes in Alloxan-Induced Diabetic Rats", Research Journal of Medicinal Plants, 2015, 9(5): 227-233. DOI: 10.3923/rjmp.2015.227.233
11. A. A. Rafiu, and C. D. Luka, "Evaluation of the Antidiabetic Property of Aqueous Extract of Ipomoea batatas Leaf on Hyperglycemia, Hyperlipidemia, Blood Electrolytes, and Enzymatic Antioxidants of Streptozotocin-Induced Diabetic Rats", J. Res. Diabetes Metab., 2018, 4(1): 21-26.
12. I. Karimzadeh, G. Haghighati, M. Ramzi, M. M. Sagheb, and K. Zomorodian. "Electrolyte disorders during vancomycin treatment in hospitalized patients at hematology-oncology wards of Namazi hospital in Shiraz", Trends in Pharmaceutical Sci., 2016, 2(3): 223-228.