Kocatepe Tıp Dergisi
Kocatepe Medical Journal
19:98-108/Temmuz/2018

ARAŞTIRMA YAZISI / RESEARCH ARTICLE

KADMİYUMA MARUZ BIRAKILAN DİYABETİK RATLARA ALFA LİPOİK ASİT VE İNSÜLİN UYGULANMASININ OLASI DÜZENLEYİCİ ETKİLERİ

POSSIBLE REGULATORY EFFECTS OF APPLICATION OF ALPHA LIPOIC ACID AND INSULIN AGAINST CADMIUM EXPOSED DIABETIC RATS

Neşe KILIÇ[1], Fahrettin AKYÜZ[1], Neslihan TEKİN[2]

[1]Department of Medical Biochemistry, Faculty of Medicine, Eskisehir Osmangazi University
[2]Department of Biotechnology and Molecular Biology, Faculty of Science and Letters, Aksaray University

ÖZ

AMAÇ: Kadmiyuma (Cd) çevresel maruziyet hiperglisemi ve azalmış serum insülini ile ilişkilidir. Bu çalışma, Cd'ye maruz kalmış diyabetik ratlarda Lipoik Asit (LA) ve insülinin glikolitik enzimler, karaciğer marker enzimleri ve lipidler üzerindeki etkilerini değerlendirmek için tasarlanmıştır.

GEREÇ VE YÖNTEM: Erkek Wistar sıçanları 7 gruba ayrıldı (n = 8). Gruplar kontrol, diyabetik kontrol, diyabet + CdCl2, diyabet + insülin, diyabet + CdCl2 + insülin, diyabet + CdCl2 + LA ve diyabet + CdCl2 + insülin + LA gruplarından oluştu. Tip 1 diyabet, 6 gruba intraperitoneal (i.p.) streptozotosin (STZ) (65 mg / kg) enjeksiyonu ile indüklendi. İnsülin (4 IU/kg/gün), insülin ile tedavi edilen gruplara subkutan (s.c.) verildi. CdCl2 (1,2 mg/kg/gün), CdCl2 ile tedavi edilen gruplara s.c. verildi. LA (100 mg/kg/gün), LA ile tedavi edilen gruplara i.p. verildi. CdCl2 ve insülin tedavisi, intraperitoneal STZ enjeksiyonundan 2 gün sonra başlatıldı ve 3 hafta sürdürüldü. Serum glukoz, AST, ALT, ALP, BUN, LDL, HDL ve TG düzeyleri, tam kan HbA1c düzeyi ve karaciğer heksokinaz (HK), piruvat kinaz (PK) ve Na+/K+ ATPaz aktivitesi değerlendirildi.

BULGULAR: Diyabetik grupta serum glukoz, HbA1c, TG, LDL, AST, ALT, ALP ve BUN düzeyleri kontrol grubuna göre daha yüksek bulundu, ancak HDL daha düşüktü. Diyabetik kontrol grubu karaciğer dokusunda Na+/K+ ATPaz, HK ve PK aktivitesi azaldı. Diabetik + CdCl2 ve Diabetik+İnsulin+CdCl2 gruplarında karaciğerde PK, HK ve Na+/K+ ATPaz aktivitesi arttı. İnsülin ve LA ile tedavi edilen gruplarda HK, PK ve Na+/K+ ATPaz aktivitelerinde diyabetik kontrol grubu ile karşılaştırıldığında artış saptandı.

SONUÇ: Bu sonuçlar insülin ve LA uygulamasının Cd ve STZ'nin neden olduğu karaciğer hasarına karşı etkili bir terapötik müdahale olabileceğini düşündürmektedir.

ANAHTAR KELİMELER: Lipoik asit, Kadmiyum, Heksokinaz, Piruvat kinaz, Na^+/K^+ ATPaz

ABSTRACT

OBJECTIVE: Environmental exposure to the cadmium (Cd), is associated with hyperglycemia and reduced serum insulin. This investigation was planned to assess the effects of Lipoic Acid (LA) and insulin on glycolytic enzymes, liver marker enzymes and lipids in Cd exposed diabetic rats.

MATERIAL AND METHODS: Male Wistar rats were separated into 7 groups (n=8 in each group). Groups were designed as control, diabetic control, diabetic + CdCl2, diabetic + insulin, diabetic + CdCl2 + insulin, diabetic + CdCl2 + LA, anddiabetic + CdCl2 + insulin + LA groups. Type 1 diabetes was established by intraperitoneal (i.p.) injection of streptozotocin (STZ) (65 mg/kg) into 6 groups. Insulin (4 IU/kg/day) was given subcutaneously (s.c.) to insulin treated groups. CdCl2 (1,2 mg/kg/day) was given s.c. to CdCl2 treated groups. LA (100 mg/kg/day) was given i.p. to LA treated groups. CdCl2, LA, and insulin treatment were started 2 days after intraperitoneal STZ injection and continued for 3 weeks. Serum glucose, AST, ALT, BUN, LDL, HDL, and TG levels and liver hexokinase (HK), pyruvate kinase (PK), whole blood HbA1c level, and Na+/K+ATPase activity were evaluated.

RESULTS: In diabetic group, serum glucose, HbA1c, TG, LDL, AST, ALT, ALP, and BUN levels were higher than control, but HDL was lower. In liver tissue, activities of Na+/K+ATPase, HK and PK activities were decreased in diabetic control group. PK, HK and Na+/K+ATPase activities were increased in liver in diabetic+CdCl2 and Diabetic+Insulin+CdCl2 groups. An increase was determined in activities of HK, PK, and Na+/K+ATPase in insulin and LA treated groups compared with diabetic control group.

CONCLUSIONS: These results suggest that application of insulin and LA could be an effective therapeutic intervention against liver injury caused by Cd and STZ.

KEYWORDS: Lipoic acid, Cadmium, Hexokinase, Pyruvate kinase, Na^+/K^+ATPase

Geliş Tarihi / Received: 18.05.2018
Kabul Tarihi / Accepted: 20.06.2018

Yazışma Adresi / Correspondence: Asst. Prof. Neslihan Tekin
Department of Biotechnology and Molecular Biology, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey
neslihan_tekin@hotmail.com

INTRODUCTION

Diabetes mellitus (DM) is one of the toughest health problems of the 21st century and influences 347 million people in the world. It is expected that this number will increase gradually and diabetes will be the 7th top reason of death worldwide in 2030.

DM is a multifaceted metabolic illness, characterized by hyperglycemia and impaired carbohydrate, protein and lipid metabolism resulting from failure in insulin action, insulin secretion or both (1). This change in the metabolism of energy molecules is due to alterations in the activities of enzymes or proteins included in glucose metabolism or in the transport of target tissues, for example muscle, liver, and adipose. Therefore, these main enzymes and proteins are significant control points in glucose homeostasis (2). The most prominent feature of diabetes is associated with abnormal glucose metabolism because of insulin deficiency. It has been revealed that the activities of the enzymes in the glycolytic and pentose phosphate pathways are reduced, while the activities of the glycogenolytic and gluconeogenic pathways are increased. For the protection of normoglycemia, coordinated regulation and integration of various metabolic pathways containing gluconeogenesis and glycolysis are required (3).

Cadmium (Cd) is a toxic transition metal considered by the Agency for Toxic Substances and Disease Registry as one of the most dangerous environmental pollutants. In the non-occupational community, the main sources of Cd intoxication are drinking water, contaminated food items and smoking tobacco. Cd is regarded greatly toxic and is increasingly bioaccumulated in organism with a biological half-life in people, which is estimated in decades (4). Chronic exposure to Cd results in metal accumulation in tissues and organs, especially in the liver and kidney and causes many histological and metabolic changes, altered gene expression, membrane damage, and apoptosis (5). Cd toxicity is furthermore connected to chancing of redox state and antioxidant system of cells, lipid peroxidation, altered expression of various proteins, and modification in protein structure (6). Epidemiologic studies indicate a positive association between high blood glucose levels and occuring of diabetes when exposed to environmental Cd. When exposed to cadmium, damage to the pancreas, liver, adrenal gland, and adipose tissue occurs. As a result of this, altered glucose uptake and/or glucose metabolism results in an increase in blood glucose. In studies, it has been shown that Cd increased hyperglycemia and nephrotoxicity in experimentally induced diabetic animals. It has also been shown in these studies that Cd decreases insulin levels and has direct cytotoxic efficacy on the pancreas (5, 7).

Type 1 diabetic patients are dependent on exogenous insulin (8). Treatment with insulin provides effective glycemic control but causes such as short shelf life, constant cooling requirement, ineffectiveness on oral administration and lethal hypoglycaemia in case of overdose limit the use of insulin (9). In recent times, an increasing concern has led to the return of traditional and alternative medicines that take the place of synthetic in the treatment of diabetes. The results obtained in the previous report clearly demonstrate that in addition to traditional antidiabetic therapy, antioxidant therapy would be beneficial (10). In this context, a series of bioactive molecules contained within vegetables, fruits, food components, and other natural resources are being continually investigated for their direct or indirect utility in inhibiting and/or administration of diabetes (3).

Lipoic acid (LA), or 1,2-dithiolane-3- pentanoic acid, is a naturally consisting dithiol substance, essential for the functions as a cofactor for some mitochondrial enzymes, cause the manufacture of ATP. It is known that LA has strong antioxidant potency and is influential in both oxidative stress inhibition and management in a number of models or clinical conditions, including diabetes (11). LA is a powerful compound with amphipathic properties, is capable of extinguishing free radical, chelating metal and regenerating other antioxidants (6).

As previous investigation, exposure to Cd is increasing depending on the development of industry and urbanization. On the other hand, there is connection with Cd exposure and diabetes as previous reports (4). Some research has demonstrated that the application of exogenous antioxidants can be supportive therapy to treat diabetes in mammals (11). In light of the above insight, the object of our study was to explore the potential influence of insulin and LA which is an antioxidant that has been clinically tested as a treatment for diabetes, against the Cd exposure in type I diabetic rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (3-4 months old; 250-300 g) were preserved under controlled conditions of temperature and humidity, with free access to water and food. The rats were obtained from Eskisehir Osmangazi University Experimental Research Center. They were maintained on a reversed 12:12 h light:dark cycle with temperature at 21±1°C.

Ethical approval: This intervention was approved by the Animal Care and Use Committee (Ethic number: 932009), Faculty of Medicine, Eskisehir Osmangazi University.

Induction of diabetes and experimental design

Fifty six rats were seperated into 7 main groups of 8 rats each (**Table 1**). Diabetes was induced in 6 group rats (n = 48) by i.p. injection of 65 mg/kg STZ (Sigma–Aldrich, St Louis, MO) in pH 4.5 sodium citrate buffer. Eight rats were injected with buffer, which used as a healthy control. Two days after treatment with STZ, blood glucose levels of overnight fasting rats were monitored in samples obtained from the tail vein of rats using accutrend strips. Rat whose blood glucose levels exceeded 12 mM were considered diabetic (12). Insulin (4 IU/day) was given s.c. to the rats for 3 weeks after 2 days the injection of STZ. The rats were administered with CdCl2 (1,2 mg/kg/day, s.c.) and LA (100 mg/kg/day, i.p.) for five times a week during 3 consecutive weeks after 2 days the injection of STZ (**Table 1**).

Table 1: Experimental groups, administered substances with the amounts and applications

Experimental Groups (n=8)	Applications
Control Group	sodium citrate buffer (i.p.)+ Physiological Saline (s.c.)
Diabetic Control	STZ (65 mg/kg, i.p.) + Physiological Saline (s.c.)
Diabetic+Insulin	STZ (65 mg/kg, i.p.) + Insulin (4 IU/day, s.c.)
Diabetic +$CdCl_2$	STZ (65 mg/kg, i.p.) + CdCl2 (1,2 mg/kg/day, s.c.)
Diabetic +Insulin+$CdCl_2$	STZ (65 mg/kg, s.c.) + Insulin (4 IU/day, s.c.) + CdCl2 (1,2 mg/kg/day, s.c.)
Diabetic +$CdCl_2$+Lipoic Acid	STZ (65 mg/kg, i.p.) + CdCl2 (1,2 mg/kg/day, s.c.) + Lipoic Acid (100 mg/kg/day, i.p.)
Diabetic +$CdCl_2$+Insulin+Lipoic Acid	STZ (65 mg/kg, i.p.) + CdCl2 (1,2 mg/kg/day, s.c.) + Insulin (4 IU/day, s.c.) + Lipoic Acid (100 mg/kg/day, i.p.)

Sample preparations

At the end of the experimental period, rats were killed by cervical dislocation following ketamine and xylazine anesthesia. Blood was collected by cardiac puncture, before the cervical dislocation. Blood samples were seperated into two different tubes, one tube for biochemical analysis in serum and the other tube which is containing EDTA for the measurment of HbA1c. Serum was collected after 3500 g centrifugation at 4 °C for 10 min to determine glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), blood Urea Nitrogen (BUN), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglyceride (TG) levels. The other whole blood samples were used for HbA1c analysis. Liver tissue samples were cleaned using an ice-cold solution of isotonic NaCl at ice cold for the removal of bloody spots, and then they were dried with blotting paper. Liver tissue were seperated into three parts for determine the enzyme activities and kept at −80 °C until analysis.

Determination of Biochemical Parameters

Serum glucose, AST, ALT, ALP, BUN, LDL, HDL, and TG levels were measured with a Roche Diagnostic Modular Analyser using Cobas Roche/Hitachi kit and and expressed as mg/dL, U/L, U/L, U/L, mg/dL, mg/dL, mg/dL, and mg/dL, respectively. HbA1c level was measured via Cobas Roche/Hitachi kit via Hithachi 911 Modular Analyzer and specified as %.

Determination of Hexokinase Activity

To determine the hexokinase (HK) activity, liver tissues were homogenized in 3 mM sodium phosphate buffer (containing 5 mM EDTA and 5 mM β- mercaptoethanol, pH 7.0) and centrifuged at 30000 g for 30 min (13). HK activity was measured spectrophotometrically by the method described by Beutler (14). Protein content was determined using the Biuret method (15). Results were expressed as units per milligram protein.

Determination of Pyruvate Kinase Activity

To determine the PK activity, liver tissues were homogenized in 50 mM Tris HCl buffer (containing 0,1 mM EDTA, pH 7.6) and centrifuged at 8000 g for 30 min (16). PK activity was assayed by the method described by Beutler (14). Protein content was determined using the Biuret method (15). Results were specified as units per milligram protein.

Determination of Na+/K+ATPase Activity

To determine Na+/K+ATPase activity, liver tissues were homogenized in 10 mM Tris HCl buffer (containing 1 mM EDTA and 0,25 M sucrose, pH 7.6) and centrifuged at 20000 g for 20 min (17). The activities of Na+/K+ATPase was assayed by the method described by Matteucci (12, 18). Protein content in the tissue homogenate was determined using the Biuret method (15). Results were specified as units per milligram protein.

Statistical analysis

Statistical Package for Social Sciences version 15.0 software was used for statistical analysis. Statistical differences between control and experimental groups were determined by one-way analysis of variance followed by Tukey post hoc comparison test. Data are presented as mean ± SD. A p values less than 0.05 were found as statistically significant.

RESULTS

Serum glucose, HbA1c, and BUN levels

The glucose, HbA1c, and BUN levels were significantly elevated in the diabetic control in comparison with the control group ($p<0.001$, $p<0.001$, $p<0.001$). Glucose level was significantly increased in the serum of diabetic control compared with the other groups ($p<0.001$). Glucose level was decreased diabetic+CdCl2+LA and diabetic+CdCl2+insulin+LA groups when compared to the diabetic+insulin group ($p<0.001$, $p<0.05$). The HbA1c levels were significantly increased in the diabetic control group in comparison with the diabetic+insulin, diabetic+CdCl2, diabetic+insulin+CdCl2, diabetic+CdCl2+LA, and diabetic+CdCl2+insulin+LA groups ($p<0.05$, $p<0.05$, $p<0.001$, $p<0.001$, $p<0.001$). The HbA1c level was decreased diabetic+CdCl2+LA group when compared to the diabetic+insulin group ($p<0.01$). BUN levels in diabetic+CdCl2+LA and diabetic+CdCl2+insulin+LA groups were significantly lower than diabetic group ($p<0.01$, $p<0.001$,) (**Table 2**).

Table 2: Serum glucose, HbA1c, creatinine, and BUN levels (mean ± SD)

	Glucose(mg/dL)	HbA1c(%)	BUN(mg/dL)
Control	147±11,6	3,76±0,28	20±2,26
Diabetic control	532±32,4**	6,55±0,21**	36,38±3,54**
Diabetic+Insulin	388±18,2**,+++	6,08±0,29**,+	33,38±4,50**
Diabetic +$CdCl_2$	456±28,9**,+++	6,11±0,23**,+	38,75±1,23**
Diabetic +Insulin+$CdCl_2$	416±50,9**,+++	5,80±0,24**,+++	32±4,69**
Diabetic +$CdCl_2$+LA	123±15,2+++,aaa	5,52±0,31**,+++,aa	29±4,10**,++
Diabetic +Insulin+$CdCl_2$+LA	333±37,8**,+++,a	5,70±0,20**,+++	26,13±2,47*,+++,***,aa

* p<0.05; ** p<0.001; (compared to control)

+P<0.05; ++P<0.01; +++P<0.001 (compared to diabetic control)

aP<0.05; aaP<0.01; aaaP<0.001 (compared to Diabetic+Insulin)

***p<0.05; (compared to Diabetic+Insulin+$CdCl_2$)

Serum AST, ALT, and ALP levels

We determined increased AST ($p<0.001$), ALT ($p<0.001$), and ALP ($p<0.001$) levels in all experimental groups when compared to control group. We found increased AST ($p<0.001$) and ALT ($p<0.001$) levels in the diabetic+CdCl2 group when compared to the other experimental groups. CdCl2 treatment significantly increased the AST levels in diabetic+CdCl2+LA groups when compared to diabetic control group ($p<0.001$). AST level was lower in diabetic+insulin than diabetic control,

diabetic+insulin+CdCl2, diabetic+CdCl2+LA, and diabetic+CdCl2+insulin+LA groups (p<0.01, p<0.001, p<0.001). We determined increased ALT levels (p<0.001) in the diabetic control group when compared to the diabetic+insulin, diabetic+insulin+CdCl2, diabetic+CdCl2+LA, and diabetic+CdCl2+insulin+LA groups (p<0.001, p<0.001, p<0.001, p<0.001). The ALT level was decreased in both diabetic+insulin and diabetic+insulin+CdCl2 groups as compared with the diabetic+CdCl2+LA and diabetic+CdCl2+insulin+LA groups (p<0.001, p<0.001). We found increased ALP levels (p<0.001) in the diabetic control group when compared to the other experimental groups. The ALP level was also higher in diabetic+CdCl2 group than in diabetic+insulin+CdCl2 and diabetic+CdCl2+insulin+LA groups (p<0.001, p<0.001) (**Table 3**).

Table 3: Serum AST, ALT, and ALP levels (mean ± SD)

	AST(U/L)	ALT(U/L)	ALP(U/L)
Control	142±4,4	51±6,9	278±29,9
Diabetic control	231±5,6*	158±13,5*	2126±43,4*
Diabetic+Insulin	216±3,2*,+,**	116±3,9*,++,**	1922±72,6*,++,
Diabetic+$CdCl_2$	510±9,1*,++	194±5,8*,++	1688±63,4*,++
Diabetic+Insulin+$CdCl_2$	236±10,4*,**,***,+++	126±4,7*,++,**,+++	1271±45,8*,++,**
Diabetic+$CdCl_2$+LA	360±9,0*,++,**,***	71±4,8*,++,**,***	1626±72,7*,++
Diabetic+Insulin+$CdCl_2$+LA	226±8,6*,**,***,+++	85±5,1*,++,**	1308±81,8*,++,**

* p<0.001 (compared to control)

+P<0.01; ++P<0.001(compared to diabetic control)

**p<0.001 (compared to Diabetic+$CdCl_2$)

***p<0.001 (compared to Diabetic+Insulin)

+++P<0.001 (compared to Diabetic+$CdCl_2$+LA)

Serum LDL, HDL, and TG levels

The LDL and TG levels were significantly elevated in the diabetic control group in comparison with the control group (p<0.001, p<0.05). We determined increased TG level in the diabetic control group when compared to the diabetic+insulin, diabetic+insulin+CdCl2 (p<0.001, p<0.001). We found decreased TG level in the diabetic+CdCl2+LA groups when compared to the control (p<0.001). We determined increased LDL level in the diabetic control group when compared to the diabetic+insulin, diabetic+insulin+CdCl2, diabetic+CdCl2+LA, and diabetic+CdCl2+insulin+LA groups (p<0.001, p<0.001, p<0.01, p<0.001).

We found decreased LDL level in the diabetic+insulin group when compared to diabetic+CdCl2 and diabetic+CdCl2+LA groups (p<0.05, p<0.05). The HDL level was decreased in the diabetic control group in comparison with the control group (p<0.001). We determined decreased HDL level in the diabetic+CdCl2, diabetic+insulin+CdCl2, diabetic+CdCl2+LA, and diabetic+CdCl2+insulin+LA groups when compared to the diabetic control group (p<0.001, p<0.001, p<0.001, p<0.001) (**Table 4**).

Table 4: Serum LDL, HDL, and TG levels (mean ± SD)

	LDL(mg/dL)	HDL(mg/dL)	TG(mg/dL)
Control	7,25±1,03	39,5±1,85	64±3,06
Diabetic control	11,75±1,66***	33±1,92***	75±3,02*
Diabetic+Insulin	7,50±0,92+++,a	34,13±1,80***,a	226±5,99***,+++
Diabetic+$CdCl_2$	9,75±0.70***,++	25,63±1,40***,+++*	62±3,10##
Diabetic+Insulin+$CdCl_2$	7,88±1,35a	26,63±1,68***,+++,##	202±12,95***,+++,aaa, ##
Diabetic+$CdCl_2$+LA	9,38±0.51**,++,#,###	26,25±1,49***,+++,##	44±3,19***,+++,aaa, ##
Diabetic+Insulin+$CdCl_2$+LA	7,75±0,88+++,aa, ###	24,5±1,77***,+++,##	71±4,14+++,##

* p<0.05; ** p<0.01; *** p<0.001 (compared to control)

+P<0.05; ++P<0.01; +++P<0.001 (compared to diabetic control)

aap<0.05; aap<0.01; aaap<0.001 (compared to Diabetic+$CdCl_2$)

#p<0.05; ##p<0.001 (compared to Diabetic+Insulin)

###p<0.001 (compared to Diabetic+Insulin+$CdCl_2$)

Na+/K+ATPase, hexokinase, and pyruvate kinase activities in liver

The liver Na+/K+ATPase activitiy was decreased in the diabetic group and diabetic+CdCl2+insulin +LA group when compared to the control group (p <0.001, p<0.05) **(Table 5).** The Na+/K+ATPase activitiy was higher in diabetic+insulin+CdCl2 and diabetic+CdCl2+LA groups than in control group (p<0.001, p<0.001). We determined increased Na+/K+ATPase activitiy in the diabetic +CdCl2, diabetic+insulin+CdCl2, diabetic+CdCl2 +LA, and diabetic+CdCl2+insulin+LA groups when compared to the diabetic group (p<0.001, p<0.001, p<0.001). The Na+/K+ATPase activitiy was decreased in diabetic+insulin group as compared with the diabetic+CdCl2, diabetic +CdCl2+LA, and diabetic+CdCl2+insulin groups (p<0.01, p<0.001, p<0.001). Activity of Na+/K +ATPase was decreased in the diabetic+CdCl2 group in comparison with the diabetic+CdCl2+LA, and diabetic+CdCl2+insulin groups (p<0.01, p<0.001) **(Table 5).**

We determined decreased HK activity in the diabetic control group when compared to the control group (p<0.001). The HK activitiy was lower in diabetic control group when compared

to the other groups ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$). HK activity was lower in diabetic+insulin group than diabetic+insulin+CdCl2, diabetic+CdCl2+LA, groups ($p<0.01$, $p<0.001$) (**Table 5**).

Table 5: Liver HK, PK, and Na+/K+ ATPase activities (mean ± SD)

	Hexokinase Activity(U/mg protein)	Pyruvate Kinase Activity(U/mg protein)	Na^+/K^+ ATPase Activity(U/mg protein)
Control	5,79±0,32	0,122±0,006	0,791±0,06
Diabetic control	1,74±0,23***,+	0,097±0,005***	0,641±0,08***
Diabetic+Insulin	3,27±0,12***,+	0,126±0,006+	0,696±0,08
Diabetic+$CdCl_2$	3,26±0,16***,+	0,111±0,008**,+,+++,#	0,826±0,06+,++
Diabetic+Insulin+$CdCl_2$	3,97±0,20***,+,++	0,147±0,006+,+++,aaa	0,995±0,06***,+,+++,aaa
Diabetic+$CdCl_2$+LA	7,90±0,25***,+,+++	0,095±0,005***,+++,aaa,#	1,130±0,04***,+,+++,aa
Diabetic+Insulin+$CdCl_2$+LA	2,44±0,13***,+,+++	0,101±0,004***,+++,a	0,678±0,07*

* p<0.05; ** p<0.01; *** p<0.001 (compared to control)

+P<0.001; (compared to diabetic control)

[a]p<0.05; [aa]p<0.01; [aaa]p<0.001 (compared to Diabetic+$CdCl_2$)

++p<0.01; +++p<0.001 (compared to Diabetic+Insulin)

#p<0.05 (compared to Diabetic+Insulin+$CdCl_2$)

The PK activity decreased in diabetic+CdCl2, diabetic+CdCl2+LA, and diabetic+insülin+CdCl2+LA group when compared to the control group ($p<0.01$, $p<0.001$, $p<0.001$). We determined increased PK activity in the diabetic+insulin+CdCl2 group when compared to the control group ($p<0.001$). The PK activities increased in diabetic+insulin, diabetic+CdCl2, and diabetic+insulin+CdCl2 groups as compared with diabetic+CdCl2 ($p<0.001$, $p<0.001$, $p<0.001$). The PK activities were significantly increased in the diabetic+insulin group in comparison with the diabetic+CdCl2+LA, and diabetic+CdCl2+insulin+LA groups ($p<0.001$, $p<0.001$) (**Table 5**).

DISCUSSION

There is little information about the evolution and complications of metabolic disorders caused by modern environmental health hazards. Cd has in recent times appeared as a most important concern not only in environmental toxicology but also in metabolic illnesses like diabetes and its complications (19). In the current study, we explored the possible ameliorative effects of combine administration of LA and insulin against experimental Cd exposed diabetic rats. For this reason, serum levels of lipids, liver marker enzymes, liver HK, PK, and Na+/K+ATPase activities were evaluated in this study.

STZ was used in our study for induction of experimental diabetes in rats. STZ-induced diabetes is caused by the specific destroy of the pancreatic islet β-cells leading insulin deficiency and hyperglycaemia. The mechanism involved in the induction of STZ induced diabetes is related to the excessive production of free radical causing toxicity in pancreatic cells which decreases the release and the synthesis of insulin, while influencing organs for example, kidney, hematopoietic system, and liver (20). In our study, the blood glucose levels of diabetic rats increased. Increased glucose level is mainly the outcome of the disruption of either peripheral or liver tissues to metabolize glucose and the activation of gluconeogenesis in the kidney and the liver (21).

On the other hand, epidemiological studies show that there is a positive relationship among exposure to environmental pollutants Cd and the frequency and intenity of diabetes. In experimental studies, it has been determined that Cd application caused the diabetogenic effects in both acute and subchronic exposure model (22). For instance, in one study, Cd was shown to elevate plasma glucose levels when compared non-fasted rats (22, 23). Additionally, Cd has been demonstrated to aggravate diabetic hyperglycemia. In the alloxan-induced diabetic animals exposed to cadmium, the fasting blood glucose level was found to be 4-fold higher, whereas in the alloxan-alone-treated animals only a 2-fold increase was detected (22, 24). In a distinct research study operating STZ-induced diabetic animals, determined that fasting blood glucose levels were 5 fold upper in the diabetic animals. In this study, no additional effect on blood glucose levels was observed in diabetic rats given Cd with drinking water for 75 days (22, 25). On the contrary, in our study, the level of glucose decreased in Cd treated diabetic rats according to the diabetic group. In some studies, blood glucose reduction was detected in Cd-administered groups (26). Some studies have also presented that heavy metals can reduce the glycogen reserves in fish and invertebrates by affecting the activities of enzymes involved in carbohydrate metabolism. It has been stated that the reduction in blood glucose may be due to a decrease in the liver glycogen concentration

or impairment of intestinal glucose absorption (27). This might suggest that Cd may alter blood glucose levels and glycogen reserves by affecting the activities of liver enzymes involved in carbohydrate metabolism such as gluconeogenesis and glycolysis.

Significant reductions in serum glucose levels were observed after LA was administered in studies performed (28). Gradually the ability of different LA formulas to enhance glucose uptake and glycogen synthesis in both in vitro and animal models has been tested. The molecule tries to improve insulin sensitivity by increasing glucose uptake and modulating signal transduction (29).

Early research has shown that LA increases insulin sensitivity and ameliorates impaired glucose tolerance. When rats are given LA, insulin-stimulated glucose transport increases throughout the body and skeleton (30). Numerous mechanisms have been suggested to demonstrate the hypoglycemic effect of LA, such as the inhibition of hepatic gluconeogenesis, the accelerated use of glucose by peripheral tissues through the translocation of glucose carriers in plasma membranes, autophosphorylation of insulin receptors and increased insulin sensitivity due to the oxidation of thiol groups in the beta subunit of the insulin receptor by the oxidized form of LA. It has also been reported that LA decreases the gluconeogenic enzyme activities, thereby lowering blood glucose and stimulating glycolysis (28). In this study, LA treatment decreased the glucose level. This might propose that LA can be inhibit harmful effect of STZ on blood glucose. Our outcomes support that antioxidant supplementation has a positive effect in regeneration of pancreatic islet cells, increasing insulin release and reducing blood glucose concentration in STZ-induced diabetic rats. On the other hand, glucose level was decreaced in diabetic+CdCl2+LA group compared to the diabetic+CdCl2+Insulin+LA group. According to these data, the administration of LA alone has been shown to have a more beneficial effect than the combination with insulin.

In general, glycosylated hemoglobin (HbA1c), advanced glycation end products formation, elevated blood glucose levels, decreased insulin levels, and body weight loss are characterized by STZ-induced diabetes. HbA1c values were reported to be close to normal after insulin treatment. Also, it was reported that LA treatment reduced blood glucose and HbA1c levels (31). It was found that HbA1c increased in diabetic condition and the quantity of increase was directly proportional to the fasting blood glucose level (32). We determined increased HbA1c level in all experimental groups when compared to control group. According to the diabetic control group, there was a significant reduction in diabetic+insulin, diabetic+CdCl2, diabetic+insulin+CdCl2, diabetic+CdCl2+LA, and diabetic+insulin+CdCl2+LA groups.

DM is usually related to irregular lipid metabolism. Hyperlipidemia is a complication associated with hyperglycemia in diabetics and is characterized by changes in TG's, C, and lipoproteins (33). The current work showed important increase in the TG and LDL levels and important decrease in the HDL level in the diabetic group compared to the control rats. Our study is compatible with other studies which show abnormal lipid pattern in diabetic subjects, suggesting the reasonableness of our results.

With the administration of LA to groups, the levels of serum TG and LDL were reduced; however, no improvement in HDL in this group was observed which was comparable with diabetic group. As in our study, other studies have reported lipid lowering effects of LA. LA can improve lipid profile through decreasing hydrxoy-methyl-glutaryl-Co-A reductase activity, and/or by increasing lecithin cholesterol acyl transferase and lipoprotein lipase activities. Also LA may normalize blood and liver TG by inhibition of hepatic lipogenic gene expressions and stimulation of TG-rich lipoproteins clearance. Recently It has been proposed that probably through activation of AMP-activated protein kinase, LA causes blocking of acetyl-CoA carboxylase, leading to enhanced mitochondrial fatty acid β- oxidation

(34). As another mechanism, it has been postulated that LA, by its anti-inflammatory functions, may downregulate the endothelial lipase, which could result in improvement of HDL levels (35).

Numerous experimental and epidemiological researches further propose that chronic exposure of Cd and STZ induced changes of lipid metabolism leads to diabetic nephropathy (36). TG level was lower in diabetic+CdCl2 group as compared to diabetic control groups. The values of our work are compatible with other studies (37). However, LDL was lower for diabetic+CdCl2 group as compared to diabetic control group.

During the impairment of carbohydrate metabolism, the liver and kidneys also play vital roles in the glucose metabolism of diabetic rat. Clinically, several enzymes and substance levels are used as biochemical markers for early diagnosis of diabetes and its complications, including AST, ALT, and BUN. In our study, rats displayed liver damage as revealed by noticeably raised enzymatic activities of serum ALT and AST indicating that diabetes can cause liver damage (38). Also, increased serum analysis of BUN level in diabetic rats has been used to reflect the impaired physical status of the kidney. On the other hand, application of Cd to diabetic rats increased the AST and ALT levels. Once Cd is taken up by the cells, it induces oxidative stresses, inflammation, and lipid peroxidation thus causing liver damage (39). From our work, the administration of insulin, LA and insulin+LA to Cd exposed diabetic rats had significant effects on the suppression of ALT and AST activities as well as BUN level in serum, suggesting that insulin and LA had potential protective effects against Cd and STZ-toxicity to the liver and kidneys (38). This might be dealing with the curative influence of insulin and LA on blood glucose levels. These results propose that LA may be an influential therapeutic application against liver damage caused by Cd and STZ.

The liver is an insulin sensitive tissue that has an important role in glucose metabolism by regulating the interplay among glucose usage and gluconeogenesis. A partial or total lack of insulin production decreases PK, HK, and glucose-6-phosphate dehydrogenase activity and causes degradation of glucose metabolism, leading to disturbed peripheral glucose utilization and increased hepatic glucose production (40).

In general, elevated glucose production, reduced glycolysis and hepatic glycogen synthesis appear to be a consequence of low HK and high glucose-6-phosphatase activities in the diabetic state (33). HK is the first key enzyme of the glycolytic pathway that catalyzes the phosphorylation of glucose for its activation to glucose-6-phosphate. (41). The restoration of HK activity may play a role in normalizing glucose levels. Insufficient HK activity in diabetic state could cause reduced usega of glucose for energy manufacture. (33). Some studies have shown a decrease in HK activity in the diabetes group compare to the control (42, 43). In experimental diabetes, a relative insulin deficiency causes a decrease in the activity of this enzyme (41). In our study, decreased HK activity was determined in STZ-induced rats. This might be because the cells are not getting enough insulin. Furthermore, hepatic HK is an inducible enzyme and its synthesis is directly or indirectly induced by insulin. Glucose phosphorylation in the liver is reduced in diabetics and it returns to its normal value by insulin therapy (44). We observed increased HK activity from insulin treated groups. The significant increase in HK activity in our study indicated that the efficiency of insulin in glycemic control. Researchers have found that Cd affects the mechanisms or rates of action of certain enzymes by activation, inactivation, ligand-releasing reactions, or by mechanisms that are not yet known (26). Cd exposure has also been shown to increase carbohydrate metabolism in the liver (45). In the study performed by Sastry et al., HK activity decreased after administration of 96 hours, 60 and 120 days of Cd in the liver, while activity increased at 15 and 30 days of exposure (27). In accordance with this, in our work, HK activities were higher in Cd applicated groups when compared with diabetic control group. The LA and insulin treated Cd exposed diabetic rats

displayed increased HK activity that may cause activation of glycolysis and elevate the usage of glucose for energy manufacture. We have supplied new evidence that LA can make better glucose metabolism through the modulation of glycolytic enzymes such as HK. This work further suggested that the modest increase in HK activity in the liver improves glucose metabolism and support glucose homeostasis. PK irreversibly catalyses the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, forming pyruvate and ATP. Its altered activity in the course of diabetic states may be expected to reduce the metabolism of glucose and ATP production. Thus, the reduction in PK activity in the liver of STZ-induced diabetic rats is responsible for decreased glycolysis and enhanced gluconeogenesis, indicating that these two pathways are distorted in diabetes. (40). In our work, in accordance with this, the activity of PK was reduced in liver tissue of diabetic control group. However, administration of insulin improved the activity of PK. It can be suggested that diabetic rats treated with insulin could increase glucose utilization by increasing the activity of PK. The finding proposed that the insulin was ameliorating the glucose metabolism by elevate the use of glucose. On the other hand, researcher reported that Cd has been shown to increase PK activity depending on dose. As their report the activation of PK was seen after adrenergic stimulation associated with Na+/H+ modifier activity in vertebrate erythrocytes. Cd-stimulated signal pathways are similar to those seen after hormone treatment in the cell (46). Similarly, in our study, Cd treated group demonstrated a net increase in PK activity in liver. This increase suggests that liver cells change with Cd administration and require more energy to maintain metabolic balance. According to these results, our study had also showed that the levels of PK activity in liver was not affected from the LA treatment. Na+/K+ATPase is a membrane-bound enzyme necessary for the active transport of diverse ions, and are very sensitive to free radical reactions. These enzyme is very sensitive to structural alterations caused by lipid peroxidation. (47). The activity of Na+/K+ ATPase in STZ-induced diabetic animals is reduced in many tissues. (48). In our work, a decrease in Na+/K+ATPase activity was detected in STZ induced diabetic rats. Reduced liver Na+/K+ATPase activity may cause in the decrement of physiological function of liver tissue. However, these outcomes suggest that high glucose levels may cause a decrease in Na+/K+ATPase activity. In a study by Carageorgiou et al., Na+/K+ATPase activity was found to be increased due to long-term administration of Cd. It has been reported that metal ions can activate Na+/K+ATPase activity in both short and long term exposures. As a consequence, they have indicated that the possible mechanism of chronic adaptation induced by Cd may have developed (49). Also, in our study, treatment with Cd elevated the Na+/K+ATPase activity in liver. Insulin stimulates the Na+/K+ATPase activity. It is known that insulin causes translocation of Na+/K+ATPase from intracellular area to the surface of the cell. Many interventions have demonstrated that insulin directly improves Na+/K+ATPase activity in liver, skeletal muscle, kidney, lymphocytes, and adipocytes many other cells and organs (50). Similarly, in our study, Diabetic+CdCl2+insulin group showed increased Na+/K+ATPase activity compared with diabetic and Diabetic+CdCl2 groups. When cells were treated with insulin, the toxic effects of STZ and Cd were abolished, proposing the insulin ameliorates cell degenerations and modulate of Na+/K+ATPase activity. Treatment with LA significantly increased the Na+/K+ATPase activity in Diabetic+CdCl2+LA group. LA may be preventing the inhibition of Na+/K+ATPase activity by causing detoxification of free radicals (47).

The mechanism by which LA affects our measured parameters may involve maintaining membrane integrity or preventing the accumulation of oxidative compounds generated as metabolic by-products in the liver cell. As a result, we can postulate that LA and insulin have a curative influence in Cd exposed diabetic liver tissue, may be owing to regulation of glycolytic enzymes and lipids via antioxidant mechanism and reduction of tissue injury.

REFERENCES

1. Moodley K, Joseph K, Naidoo Y, Islam S, Mackraj I. Antioxidant, antidiabetic and hypolipidemic effects of Tulbaghia violacea Harv. (wild garlic) rhizome methanolic extract in a diabetic rat model. BMC Complement Altern Med 2015; 17;15:408.

2. Atangwho IJ, Yin KB, Umar MI, Ahmad M, Asmawi MZ. Vernonia amygdalina simultaneously suppresses gluconeogenesis and potentiates glucose oxidation via the pentose phosphate pathway in streptozotocin-induced diabetic rats. BMC Complement Altern Med 2014; 30;14:426.

3. Srinivasan S, Sathish G, Jayanthi M, Muthukumaran J, Muruganathan U, Ramachandran V. Ameliorating effect of eugenol on hyperglycemia by attenuating the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. Mol Cell Biochem 2014;385(1-2):159-68.

4. Treviño S, Waalkes MP, Flores Hernández JA, León-Chavez BA, Aguilar-Alonso P, Brambila E. Chronic cadmium exposure in rats produces pancreatic impairment and insulin resistance in multiple peripheral tissues. Arch Biochem Biophys 2015; 1;583:27-35.

5. Singh PK, Baxi D, Diwedi R, Ramachandran AV. Prior cadmium exposure improves glucoregulation in diabetic rats but exacerbates effects on metabolic dysregulation, oxidative stress, and hepatic and renal toxicity. Drug Chem Toxicol 2012;35(2):167-77.

6. Lobato RO, Nunes SM, Wasielesky W, et al. The role of lipoic acid in the protection against of metallic pollutant effects in the shrimp Litopenaeus vannamei (Crustacea, Decapoda). Comp Biochem Physiol A Mol Integr Physiol 2013;165(4):491-7.

7. Edwards JR, Prozialeck WC. Cadmium, diabetes and chronic kidney disease. Toxicol Appl Pharmacol. 2009; 238(3):289-93.

8. Szkudelski T, Szkudelska K, Anti-diabetic effects of resveratrol. Ann N Y Acad Sci. 2011 Jan;1215:34-9.

9. Abdulrazaq NB, Cho MM, Win NN, Zaman R, Rahman MT. Beneficial effects of ginger (Zingiber officinale) on carbohydrate metabolism in streptozotocin-induced diabetic rats. Br J Nutr 2012;108(7):1194-201.

10. Saravanan G, Ponmurugan P, S-allylcysteine Improves Streptozotocin-Induced Alterations of Blood Glucose, Liver Cytochrome P450 2E1, Plasma Antioxidant System, and Adipocytes Hormones in Diabetic Rats. Int J Endocrinol Metab 2013;1;11(4):e10927.

11. Al-Rasheed NM, Al-Rasheed NM, Attia HA, et al. Adverse cardiac responses to alpha-lipoic acid in a rat-diabetic model: possible mechanisms?. J Physiol Biochem 2013;69(4):761-78.

12. Tekin N, Akyüz F, Temel HE. NO levels in diabetes mellitus: Effects of l-NAME and insulin on LCAT, Na(+)/K(+) ATPase activity and lipid profile. Diabetes Metab Syndr 2011;5(4):191-5.

13. Gahr, M. Isoelectric focusing of hexokinase and glucose-6-phosphate dehydrogenase isoenzymes in erythrocytes of newborn infants and adults. Br J Haematol 1980;46(4), 529–535.

14. Beutler E, Blume KG, Kaplan JC, Löhr GW, Ramot B, Valentine WN. International Committee for Standardization in Haematology : Recommended Methods for Red-Cell Enzyme Analysis. Br J Haematol 1977; 35(2): 331–340.

15. Kingsley FGR. The direct biuret method for the determination of serum proteins as applied to photoelectric and visual colorimetry. J Lab Clin Med 1942; 27:840-845.

16. Yılmaz S, Karahan D, Kandemir FM. Effects of Some Nitrosamines on Pyruvate Kinase Activity in Tissues of Rats. Fırat Üniv Sağ Bil Derg 2008;22(3):163–168.

17. Schweitzer JB, Smith RM, Jarett L. Differences in organizational structure of insülin receptor on rat adipocyte and liver plasma membranes: Role of disulfide bonds. Proc Natl Acad Sci USA 1980;77(8):4692-4696.

18. Matteucci C, Cocci FF, Pellegrini L, Gregori G, Giampietro O. Measurment of ATPases in red cells: setting up and validation of a highly reproduciple method. Enz Protein 1995;48:115–9.

19. Anetor JI, Uche CZ, Ayita EB, et al. Cadmium Level, Glycemic Control, and Indices of Renal Function in Treated Type II Diabetics: Implications for Polluted Environments. Front Public Health 2016;13;4:114.

20. Schmatz R, Perreira LB, Stefanello N, et al. Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats. Biochimie 2012;94(2):374-83.

21. Valera A, Rodriguez-Gil JE, Bosch F. Vanadate treatment restores the expression of genes for key enzymes in the glucose and ketonebodies metabolism in the liver of diabetic rats. J Clin Invest 1993;92(1):4-11.

22. Edwards JR, Prozialeck WC. Cadmium, Diabetes and Chronic Kidney Disease. Toxicol Appl Pharmacol 2009;238(3): 289-93.

23. Bell RR, Early JL, Nonavinakere VK, Mallory Z. Effect of cadmium on blood glucose level in the rat. Toxicol Lett 1990;54:199–205.

24. Chandra SV, Kalia K, Hussain T. Biogenic amines and some metals in brain of cadmium-exposed diabetic rats. J Appl Toxicol 1985;5:378–381.

25. Bernard A, Schadeck C, Cardenas A, Buchet JP, Lauwerys R. Potentiation of diabetic glomerulopathy in uninephrectomized rats subchronically exposed to cadmium. Toxicol Lett 1991;58:51–57.

26. Ithakissios DS, Ghafghazi T, Mennear JH, Kessler WV. Effect of multiple doses of cadmium on glucose metabolism and insulin secretion in the rat. Toxicol Appl Pharmacol 1975;31(1):143-149.

27. Sastry KV, Shukla V. Acute and chronic toxic effects of cadmium on some haematological, biochemical, and enzymological parameters in the fresh water teleost fish Channa punctatus, Acta Hydrochimica Et hydrobiologica 1994;22(4):171-176.

28. Winiarska K, Malinska D, Szymanski K, Dudziak M, Bryla J. Lipoic acid ameliorates oxidative stress and renal injury in alloxan diabetic rabbits, Biochimie 2008;90(3):450-459.

29. Scaramuzza A, Giani E, Redaelli F, et al. Alpha-Lipoic Acid and Antioxidant Diet Help to Improve Endothelial Dysfunction in Adolescents with Type 1 Diabetes: A Pilot Trial. J Diabetes Res 2015;2015:474561.

30. Yang Y, Li W, Liu Y, Li Y, Gao L, Zhao JJ. Alpha-lipoic acid attenuates insulin resistance and improves glucose metabolism in high fat diet-fed mice. Acta Pharmacol Sin 2014;35(10):1285-92.

31. Maritim AC, Sanders RA, Watkins JB. Effects of α-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats, J Nutr Biochem 2003;14(5):288–294.

32. Sellamuthu PS, Muniappan BP, Perumal SM, Kandasamy M. Antihyperglycemic effect of mangiferin in streptozotocin induced diabetic rat. Journal of Health Science 2009;55(2):206-214.

33. Amalan V, Vijayakumar N, Indumathi D, Ramakrishnan A. Antidiabetic and antihyperlipidemic activity of p-coumaric acid in diabetic rats, role of pancreatic GLUT 2: In vivo approach. Biomed Pharmacother 2016;84:230-236.

34. Chen WL, Kang CH, Wang SG, Lee HM. α-Lipoic acid regulates lipid metabolism through induction of sirtuin 1 (SIRT1) and activation of AMP-activated protein kinase. Diabetologia 2012; 55: 1824-35.

35. Mirtaheri E, Gargari BH, Kolahi S, Asghari-Jafarabadi MA, Hajaliloo M. Effect of Alpha-lipoic Acid Supplementation on Serum Lipid Profile in Women with Rheumatoid Arthritis. Nutr Food Sci Res 2014;1(1): 11-18.

36. Kandasamy N, Ashokkumar N. Reno protective effect of myricetin restrains dyslipidemia andrenal mesangial cell proliferation by the suppression of sterol regulatory element binding proteins in an experimental model of diabetic nephropathy. Eur J Pharmacol 2014; 15;743:53-62.

37. Yargıçoğlu P, Agar A, Edremitlioğlu M, Kara C. The effects of cadmium and experimental diabetes on vep spectral data and lipid peroxidation. Int J Neurosci 1998;93(1-2):63-74.

38. Liu M, Song X, Zhang J, et al. Protective effects on liver, kidney and pancreas of enzymatic- and acidic-hydrolysis of polysaccharides by spent mushroom compost (Hypsizigus marmoreu). Sci Rep 2017;24(7):43212.

39. Cao Z, Fang Y, Lu Y, et al. Melatonin alleviates cadmium-induced liver injury by inhibiting the TXNIP-NLRP3 inflammasome. J Pineal Res 2017;62(3).

40. Srinivasan S, Sathish G, Jayanthi M, Muthukumaran J, Muruganathan U, Ramachandran V. Ameliorating effect of eugenol on hyperglycemia by attenuating the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. Mol Cell Biochem 2014;385(1-2):159-68.

41. Stanley Mainzen Prince P, Kamalakkannan N. Rutin Improves Glucose Homeostasis in Streptozotocin Diabetic Tissues by Altering Glycolytic and Gluconeogenic Enzymes. J Biochem Mol Toxicol 2006;20(2):96-102.

42. Rana S, Sing, R, Verma S. Protective effects of few antioxidants on liver function in rats treated with cadmium and mercury. Indian J Exp Biol 1996;34(2):177-179.

43. Rathi SS, Grover JK, Vats V. The effect of Momordica charantia and Mucuna Pruriens in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism, Phytother Res 2002;16(3):236-243.

44. Salas M, Viñuela, E, Sols A. Insulin-dependent synthesis of liver glucokinase in the rat, J Biol Chem 1963;238(11):3535-3538.

45. Newairy, A. A., El-Sharaky, A. S., Badreldeen, M. M., Eweda, S. M., Sheweita, S. A. The hepatoprotective effects of selenium against cadmium toxicity in rats. Toxicology 2007;242, (1-3), 23–30.

46. Dailianis S, Kaloyianni M. Cadmium induces both pyruvate kinase and Na+/H+ exchanger activity through protein kinase C mediated signal transduction, in isolated digestive gland cells of Mytilus galloprovincialis (L.). J Exp Biol 2004;207(10):1665-1674.

47. Sacan O, Turkyilmaz IB, Bayrak BB, Mutlu O, Akev N, Yanardag R. Zinc supplementation ameliorates glycoprotein components and oxidative stress changes in the lung of streptozotocin diabetic rats. Biometals 2016;29(2):239-48.

48. Vague P, Coste TC, Jannot MF, Raccah D, Tsimaratos M. C-peptide, Na+/K+ATPase, and Diabetes. Exp Diabesity Res 2004;5(1):37-50.

49. Carageorgiou H, Tzotzes V, Pantos C, Mourouzis C, Zarros A, Tsakiris S. In vivo and in vitro effects of cadmium on adult rat brain total antioxidant status, acetylcholinesterase, (Na+, K+)-ATPase and Mg2+ATPase activities: protection by L-cysteine. Basic Clin Pharmacol Toxicol 2004;94(3):112-8.

50. Fehlmann M, Freychet P. Insulin and glucagon stimulation of (Na+-K+)-ATPase transport activity in isolated rat hepatocytes. J Biol Chem 1981;256(14):7449-53.