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ACID DIETARY AS DIABETOGENIC RISK FACTORS

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ABSTRACT

Investigation of diabetogenic impact of acid dietary were performed on rabbits (n=72) in several groups, control and treated animal by freshly natural lemon juice (LJ) 2 and 5 ml / kg body weight once daily intragastric administered for 2 weeks. In parallel, Helichrysum *Thianschanicum* Regel soluble extract (HTRSE 2 ml / kg) was used as an antidiabetic plant. In all experiments, Insulin resistance (IR) and glucose tolerance tests (GT) were achieved.

It was found that under the influence of LJ dietary, I) pH indicator decreased respectively 6.2 % for blood and 16.9 % for urine ($P < 0001$). II) Blood glucose level and HbA1c were increased 55.0% and 51.2% respectively ($P < 0001$). III) by IR analysis, the hypoglycemia was noted 86.1% after 45 min and 84.2% after 90 min. IV) by GT test, glycaemia increased from 23.6% to 60.2% for 2ml/Kg of acid diets ($P < 0001$) as well as 37.7% to 67.2% for 5 ml/kg after 60 to 180 mins ($P < 0001$) respectively. V) After two weeks of diets, the alteration of liver and kidney biomarkers such as ALAT, ASAT, as well as uric acid, creatinine and Urea respectively were observed. VI) When the pre-diabetic animals dieted by LJ and HTRSE (pH=6.7) simultaneously, all pathologic indicators were normalized. In conclusion pre-diabetes stage as a consequence of acid dietary is associated with decrease of blood and urine pH and alteration of liver and kidney function. HTSE as non-acid dietary had shown an anti-diabetic effect.

Introduction

Diabetes mellitus (type-2) as a non-contaminable disease and is one of the leading causes of morbidity and mortality in the world [1]. More than half of diabetics have taken dietary supplements or used alternative medicine (2). Traditionally, phytotherapy is one of current methods for control of glycaemia [3]. Based on the pathophysiology of the disease, several mechanism such as inhibition of glucose absorption [4] , upregulation of glucose transporters [5], regulation of glycogen metabolism [6], insulinomimetic activity [7, 8], regulation of endogenous opioids [8,9] and antioxidant properties [10] were attributed to the plants currently used in diabetes treatments. It is evident that multiple families of plants and/or their biological active constituents could regulate the hyperglycemia via several signalization pathways [11].

In central Asia, habitually, the medical properties of the plants or their fruits were classed in the three categories, acid, neutral and alkaline. Based on the Canon of Avicenna [12], several local investigations allowed identifying the medical possession of regional plants as well as their categories [13, 14]. After them, the intensive consummation of 'acid' plant's categories induces the metabolic disorder and in consequence caused several syndromes including diabetes. Curiously, in parallel, acid dietary was currently used as antidiabetic solution for diabetes mellitus treatment. This contradiction was conducted by our group to verify the diabetogenic impact of acid dietary in animal model.

Material and Methods

1- Animal: Rabbits (n=72, wild type, aged 4 weeks) of both sexes with an average weight of 1.8-2.0 kg. The animals were divided into five groups for experimentations.

2- Fruit and plant extraction: Natural lemon juice from Citrus Limon (*Cítrus × méyeri*) was extracted and conserved at 4°C and used for three days. *Helichrysum Thianschanicum* Regel soluble extracts (HTRSE) was performed from yellow flower and green leaf of plant. 10g of the plant with 100 ml of demineralized water was boiled for 15 min (96°C) and reposed for 45 min and conserved at 4°C. Filtered hydro soluble extract were used as an anti-diabetic in our experiments. Each preparation was used only for three days and in consequence several extractions were performed.

3- Non-acid dietary: we used the *Helichrysum Thianschanicum* Regel as a non-acid dietary plant classified by Avicenna as an alkaline dietary category that grows in neutral or alkali pH. This plant is currently used in central Asia as anti-acid and anti-spasmodic for digestive problems. The hydro-soluble extract (pH 6.7) of this plant (2ml/kg/ day) was used for two weeks in pre-diabetic rabbits (n=30) induced by LJ.

4- Acid dietary: For animal experiments, the rabbits were divided into the following four; 1st group (n=10) as control animals that were in the same conditions with other content stored in the vivarium rabbits; 2nd and 3rd series (n=21 for each) were treated respectively by freshly squeezed natural LJ 2 or 5 ml / kg body weight once daily intragastric administered for 2 weeks (n=7). Among each series, Insulin resistance(IR) performed by subcutaneous injection of 0.5 ml/kg (TI 0.5 Unit of activity per kg/weight , Actrapid Human genetic engineering insulin) (n=7) and glucose tolerance (GT) test were performed by alimentation of animals with 2 grams sugar/kg (n=7). Group 4 dieted by *Helichrysum Thianschanicum* Regel soluble extract (HTRSE, 2 ml / kg) and group 5 after 14 day of diet by 5 ml of LJ, in parallel alimented by HTRSE (2 ml / kg). All experimental dietary conditions presented in table-1.

Blood glucose (mmol/L) was analyzed after 45 and 90 min. All experimental protocols were performed in accordance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe, 1986, ETS No. 123).

5- Blood analysis: All blood samples collected in heparin tubes from ear by classical methods. According of analysis, plasma or erythrocytes were separately used. Several hematologic analysis such as blood and urine pH, the level of glycolisated hemoglobin A1c (Hb-Alc, GH) (Hb-Alc,% using " Elta " set of glikogenotesta Diagnostic, Russia), glycaemia (diagnostic kits of the Vital company Saint-Petersburg), cholesterol, triglycerides, low density lipoproteins (LDL), high density lipoprotein (HDL), uric acid (UA), creatinine, urea and residual nitrogen [15] of the blood, level of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) of blood composition, total protein and albumin were analyzed. Biological parameters such as pH of blood (using an apparatus «pH Marci- 510, France) and of urine (using pH-Biokan Saint-Petersburg) were performed.

6- Statistical studies: All values reported are the average ± SEM. Statistical significance was determined using the GraphPad Prism 6.0 software (Kruskal-Wallis test) and $P < 0.05$ was considered statistically significant.

Results

1- Acid alimentation decrease blood and urinary pH: As presented in table-2, it was found that under the influence of L.J dietary, blood and urine pH modified after two weeks. Compared with the non-treated animals, after acid dietary (5ml/kg of LJ), pH indicator decreased respectively 6.2 % for blood and 16.9 % for urine ($P < 0001$). These results indicate that acid diet altered acid-base balance in blood circulation as well as in urine.

2- Acid alimentation increase glycaemia and glycosylated hemoglobin-A1c: To observe the pre-diabetic state of animals, two types of analysis were currently preconized, blood glycaemia and glycosylated hemoglobin-A1c. The result

shows in Table-3 demonstrated that the level of blood sugar increased 55.0 % and 58.1% respectively compared to control.

In parallel, glycosylated hemoglobin A1c increased from 51.1% to 72.0% in dose dependent manner of LJ diets. These results indicate that all animals which dieted by 2 dose of acid dietary (2 and 5 ml/kg of LJ) strongly perturbed glycaemia (% indicated in the table 3).

3- Tolerance to glucose decreased by acid dietary: to measures the animal body's ability for blood glucose dynamism, oral glucose tolerance test (GT) was performed. As presented in table-4, compared to control, after 60 and 180 min of oral glucose administration, glucose tolerance was reduced by increasing of glycaemia from 28% to 38% after 60 min and from 17.4% to 18.3% after 180 min in dose dependent manner of acid dietary.

As presented in figure-1, when these results compared with non-treated animals (Baseline taken as 100%), a sever GT was presented in dose and time manners, and glycaemia increased from 23.6% to 60.2% for 2ml/Kg of acid diets ($P < 0001$) as well as from 37.7% to 67.2% for 5 ml/kg after 60 to 180 mins ($P < 0001$) respectively. These results indicate alteration of blood glucose absorption in acid dietary alimentation.

4- Acid dietary induces insulin tolerance: Subcutaneous injection of insulin as presented in table-5 reduced significantly the amount of glycaemia from 13.6% after 45min to 18.1% after 90 min in control group. These amount perturbed when the animals having acid alimentation and glycaemia decreased from 4.6% to 12.3% in the animals dieted 2 ml/kg of LJ and from 5.4% to 9.4% in the animals alimented with 5mlKg LJ after 45 to 90 min respectively. When these results compared with IT of non-treated animals, significate modification was observed in animals alimented with acid dietary (Figure-2). These results are in accord with the previous observation and indicate diabetogenic effect of acid dietary.

5- Acid alimentation altered liver function: The liver has a most importance in the body physiopathology. As presented in Table-6, the acid diet induces the functional alteration in the liver. The hepatotoxic effects of acid alimentation after 2 weeks expressed by an increase level of ALAT from 36.2% to 70.6% ($P <0.001$), ASAT from 33.1% to 82.7% ($P<0.001$) in animals with 2 or 5 ml/LJ respectively. In parallel, the level of total protein ($P<0.05$) and albumin ($P<0.05$) of serum composition of the blood plasma slightly reduced.

As presented in table-7, in the result of two-week administration of acid alimentation, the level of triglycerides of blood serum increased on 25 to 35% ($P <0.0\ 01$) as well as the lipids markers such as cholesterol (16.1 to 24%), LDL (14.5 to 26.4%) and the level of HDL decreased 2.4 to 19.0% in dieted animals respectively by 2 or 5 ml/kg of L J.

6- Acid alimentation predisposed kidney physiological perturbation: In parallel, the level of uric acid, creatinine, urea and residual nitrogen composition of blood were quantified. As presented in figure-3, the dose of 2ml/kg L J increased significantly uric acid. The level of creatinine, urea and residual nitrogen was not modified. After 2 weeks of acid diet with 5ml/kg, the amount of blood uric acid, creatinine and urea pointedly increased about 25 to 30% and residual nitrogen not changed. These results suggest that acid alimentation predisposed a chronic alteration of kidney and reformed considerably the biomarkers of kidney function.

7- The extract of *Helichrysum Thianschanicum* Regel normalized pre-diabetic stage: As presented in table-8, after 14 days treatments of the pre-diabetic rabbits by HTE, tested biomarkers in blood as well as liver and kidney having tendency to normalization. The blood pH was increased and glycaemia significantly decreased ($p<0.05$). In contrast glycosylated hemoglobin not changed. Hepatic biological parameters such as ALAT ($p<0.01$), ASAT ($p<0.01$), total and conjugated bilirubin ($p<0.05$) remarkably changed and approached to normal values. Uric acid ($p<0.\ 01$) and other functional markers of kidney such as urea ($p<0.001$) and creatinine ($p<0.05$) significantly decreased. These results indicated that oral administration of soluble extract of Helichrysum Thianschanicum Regel neutralized pre diabetogenic effect of LJ dietary. Interestingly, all animals treated by HTRSE compared to diabetics animal showed significant diminution of cholesterol ($p<0.01$) and alkaline phosphatase ($p<0.001$). In the treated animal compared with nondiabetic, the cholesterol level also considerably decreased. These results indicate the anti-diabetic property of HTRSE in pre-diabetic rabbits and also suggest the hepato-protector and anti -cholesterol effect of Helichrysum Thianschanicum Regel soluble extracts.

Discussion

In this study, we described the effect of acid diets on the induction of prediabetes stage in animal model and demonstrated that permanent lemon juice alimentation actively changed glycaemia and glycosylated Hb-A1c in blood. Overall, very similar laboratory data such as modification of pH of blood and urine into acidic side and nephro- hepato-toxic markers perturbation were observed in each groups alimented at daily oral (per os) doses for 14 days.

Our results showed that acid dietary increased significantly serum uric acid and urea. These results may be a consequence of chronic acidosis due to acid dietaries, because when the animals dieted by Helichrysum Thianschanicum Regel extracts, simultaneously glycaemia and both renal and kidney pathogenic indicators including blood pH and uric acid having to normalize. In the other studies, we used several plants extracts such as Nigella sativa L , seedlings Triticum vulgare Vill and Hordeum vulgare L for their anti-diabetes effect and similarly results to HTRSE were obtained (results not shown).

Consequently, the boundaries between medical and diabetogenic properties of aliments mainly depend on such endogenous factors as the level of acid-alkaline resources or acid-base balance [13, 14]. The impact of acid alimentation matters consummation independently to their diabetogenesis etiology causes several disorders in body functions especially in that the organism that cannot change the level of the pH. In the other study several acid alimentations such as marinated tomatoes-Solanum Lycopersicon esculentum Mill, Gruel based on 5 ml/kg. Burm. Fil, «Guraob» - juice of unripe fruit (1 and 5ml/kg) of grapes – Vitis vinifera L. Broth (1:10) of fruit of hips (2 and 5 ml/kg) of dog – Rosa canina L., Aerated beverages of Coca-Cola (5 ml/kg). Ammonium nitrate (NH_4NO_3) (100 mg/kg), were tested for their diabetogenic activity. In all experiments, we observed the same results as well as alteration of liver and kidney (results not shown).

Recently, the relationship between serum uric acid concentration and insulin resistance was reported [16]. In previous epidemiological studies, serum uric acid (an end product of purine metabolism) has been shown to be associated with an increased risk of hypertension [17, 18], cardiovascular disease [19, 20], and chronic kidney disease [21]. Also, an elevated level of uric acid reported as a risk factor for peripheral arterial disease [22] and insulin resistance, a consequence of the metabolic syndrome [15]. However, the putative association between serum uric acid levels and diabetes mellitus is not clear. Some studies reported that there is a positive association between high serum uric acid levels and diabetes [23,-28], whereas other studies reported no association [29], or an inverse relationship [30, 31]. The results presented in this study, emphasized the parallel frequency of prediabetes stage and the initial level of uremia. A hyperuricemia associated with decrease of blood pH, may be a co-factor to develop a stage of endothelial cell system perturbation and acidosis [13].

Previously by similar experiments, we reported the influence of acid diets on the endothelial dysfunctionality that provokes metabolic disorders [13] by liver dysfunctionality and alteration of lipoprotein and triglycerides. Dyslipidemia is considered one of the main indicators of the metabolic syndrome [32, 33].

The molecular mechanism of cell dysfunction in the presence oh hydrogen ions or urea in diabetic patients is not clear. High concentration of hydrogen ion (proton) as well as urea destabilizes many macromolecular structures and inhibits their functions such as ligand binding [34]. The deleterious effect of urea on proteins has been counteracted by osmolytes such as glycerophosphocholine (GPC). In humans, osmolytes have a particular importance in the renal medulla and protect the medulla from high concentration of NaCL and Urea. It was shown that a high extracellular concentration of urea or NaCl causes these cells to accumulate large amounts of GPC. Metabolomic screening of plasma from nondiabetic subjects identified α-hydroxybutyrate (α-HB) and linoleoyl-glycerophosphocholine (L-GPC) reported as joint markers of insulin resistance (IR) and glucose intolerance in early stage of the development of dysglycemia and type 2 diabetes [35, 36]. Injurious effect of hydrogen ions on the osmolytes expression by the cells may be caused the liver and kidney cells dysfunction.

Diabetogenic property of acid dietary was first proposed by Avicenna (980–1037, in medieval Persia) provided a detailed account on diabetes mellitus in "The Canon of Medicine" [12]. Like Aretaeus (Aretaeus of Cappadocia), an ancient Greek physician before him [37], Avicenna recognized primary and secondary diabetes. In the Canon of Medicine, Avicenna proposed a number of potentially diabetogenic alimentation in that the majorities are the acid dietary. In counterpart, he proposed a list of the most promising anti-diabetic medicinal plants (table-9) as an alkaline dietary [12].

According to Avicenna, for each person, type of the body is in harmony with their sensibility to acid, neutral and alkaline alimentation. In consequence tolerance to different diets is varied. These internal properties of the bodies were learned by Avicenna as temperaments (mizâj) and correspond to both kinds of cold (for not supporting acid alimentation) and hot (for not supporting alkaline alimentation). The balance of cold/hot was presented as a biological law in the Canon of medicine [12]. Disruption of this balance may conduit to metabolic syndrome. In consequence acid dietaries have a diabetogenic risk in cold kind persons.

In conclusion, we demonstrated diabetogenic potential of acid dietary and confirmed Avicenna hypothesis. These results indicated the wrong proposition of acid dietary for treatment of diabetes in central Asia. Taken as whole, these results show a diabetogenic consequence of acid diets by direct parallelism between decreases of blood and urine pH and the increased levels of blood sugar as well as glycated hemoglobin. Because of liver and kidney dysfunctionality, the possibility of metabolic syndrome induced by acid dietary may be taken in consideration.

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Table 1. Experimental dietary conditions.

	group-1	group-2			group-3			group-4	group-5
	Ctrl-1	test	IRT	GTT	test	IRT	GTT	Ctrl-2	test
N	10	7	7	7	7	7	7	10	10
L J-2ml	-	+(14 d)	+(14 d)	+(14 d)	-	-	-	-	-
L J-5ml	-	-	-	-	+(14 d)	+(14 d)	+(14 d)	-	+(28 d)
HTSE	-	-	-	-	-	-	-	+(14 d)	+(14 d)

Table-2: The blood and urine pH in the experimental rabbits.

Subject of study	pH levels after oral administration at doses of LJ			
	2 ml/kg		5 ml/kg	
	initial	14 day	initial	14 day
pH (blood)	7,46±0,02 100%	7,40±0,03 - 0,8%	7,46±0,02 100%	7,0±0,04 -6,2%
Ph (urine)	7,3+0,02 100%	6,8+0,04 -6,8%	7,3+0,02 100%	6,06±0,04 - 16.9%

Table-3 : The glycaemia and HbA1C amount in the experimental rabbits .			
Data after 14 days of diets by LJ	**control**	**2 ml/kg**	**5 ml/kg**
Glycaemia mmol/L	4,85±0,02 100%	7,52±0,03 +55,0%	7,67±0,02 +58,1%
HbA1c %	4,30±0,05 100%	6,50±0,04 +51,1% (+51,2%)	7,41±0,03 +72,0% (+72,3%)

Table-4 : Oral glucose tolerance test (OGTT) in experimental rabbits .			
series of experiments	**Gain of glycemia (mmol/l) by oral glucose administration**		
	Baseline taken as 100%	**60 min**	**180 min**
Ctrl	4.80±0.03 100%	7.7±0.02 +60.4%	5.43±0.03 +13.1%
LJ 2 ml/kg	7.41±0.03 100%	9.52±0.05 +28.4%	8.70±0.04 +17.4%
LJ 5 ml/kg	7.67±0.03 100%	10.6±0.02 +38.2%	9.08±0.03 +18.3%

Table-5 : insulin tolerance test (ITT) in experimental rabbits				
№	**series of experiments**	**Blood glucose %, after**		
		Initial 100%	45 min.	90 min.
1.	Control Group	4.4±0.07 100%	3.8±0.01 -13.6%	3.6±0.07 -18.1%
2.	LJ, 2 ml/kg	6.5±0.04 100%	6.2±0.06 - 4.6%	5.7±0.04 -12.3%
3.	LJ, 5 ml/kg	7.4±0.03 100%	7.0±0.02 - 5.4%	6.7±0.04 - 9.4%

Table-6: The hepatic parameters modification in the experimental rabbits .			
Data after 14 days of diets	**control**	**2 ml/kg**	**5 ml/kg**
ALAT nmol/L	131,0±0, 5 100%	178,5±0, 5 +36,2% P<0,002	223,5±0, 7 +70,6% P<0,001
ASAT nmol/L	62,1±0, 6 100%	82,7±0, 4 +33,1% P<0.01	113,5±0, 4 +82,7% P<0.001
Total protein mmol/L	47,7±0, 2 100%	44,7±0, 9 -6,3% P›0.1	37,7±0, 6 -21,0% P›0.05
Albumin mmol/L	31,3±0, 2 100%	30,6±0, 5 -2.2% P›0.1	35,8+0,2 -14,4% P›0.05

Table-7: The blood's lipid modification in the experimental rabbits .			
Data after 14 days of diets	**control**	**2 ml/kg**	**5 ml/kg**
Cholesterol mmol/L	1,24±0, 7 100%	1,44±0, 8 16.1%	1,54±0, 5 +24,1%
Triglycerides mmol/L	101,2±0,7 100%	126,2±0, 8 +24.7%	137,2±0, 1 +35.6%
LDL mg%	220±0, 5 100%	252±0, 1 +14.5%	278±0, 3 +26.4%
HDL mmol/L	9,76±0, 5 100%	9,52±0, 8 - 2.45%	7,9±0, 7 - 19.0%

Table-8: The extract of *Helichrysum Thianschanicum* normalized pre-diabetic stage			
Data	**Intact**	**Control**	**Helichrysum thianschanicum regel.**
Blood pH	7,3±0,01 100%	7,0±0,04 -4.1%	7,36±0,04 +5.2%
Glucose mmol/l	5,0±0,06 100%	6,8±0,06 +36%* P‹0.01	5,5±0,01 -19.1%** P‹0.05
glycosylated hemoglobin %	4,3%±0,03 100%	7,5%±0,01 +74.4% P‹0.001	7,3%±0,02 -2.7% P›0.05
Total protein (g/l)	64,2±0,03 100%	54,1±0,01 -16% P›0.05	60,7±0,01 +12% P‹0.05
Albumin g/l	36,8±0,02 100%	34,1±0,01 -7,3% P›0.05	36,5±0,06 +7.04% P›0.05
ALAT nmol/s×l	150,3±0,02 100%	249,6±0,05 +66.06% P‹0.001	183,2±0,03 -27% P‹0.01
ASAT nmol/s×l	127,1±0,05 100%	223,1±0,04 +76% P‹0.001	189,2±0,03 -15.2% P‹0.01
Cholesterol mmol/l	3,3±0,01 100%	3,9±0,04 +18.2% P‹0.05	2,5±0,01 -35.9% P‹0.01
Alkaline phosphatase E/l	81,7±0,01 100%	164±0,04 +100.7% P‹0.001	108±0,01 -34% P‹0.001
Total bilirubin mkmol/l	13,12±0,03 100%	21,33±0,05 +62.6% P‹0.001	16,6±0,01 - 22.2% P‹0.05
Conjugated bilirubin mkmol/l	8,03±0,06 100%	16,6±0,05 +106.7% P‹0.001	13,5±0,05 -18.7% P‹0.05
Urea mmol/l	4.33±0,04 100%	6.31±0,04 45,7% P<0.01	3.92±0,04 -37,8% P<0.001
Uric acid mmol/l	200±1,0 100%	332±8,0 +66% P<0.001	206±4,0 - 37,9% P<0.01
Creatinine mkmol/l	30.9±1,8 100%	37.3±0.05 20,7% P<0.05	35.7±0,04 - 4,3% P<0.05

Note: * - compared to the original (Baseline taken as 100%) ** - In comparison with control series.

Table-9: the medicinal plants with anti-diabetic effects listed in the Avicenna canon of medicine.
1- Artemisia dracunculus L.
2- Helichrysum arenarium Moench.
3- Vitis vinifera L.
4- Geranium collinum Stephan.
5- Gypsophila Plantarum L.
6- Origanum vulgare L.
7- Hypericum scabrum L.
8- Rubia tinctorum L.
9- Mentha arvensis L.
10- Triticum vulgare Vill.
11- Rhodiola quadrifida Fisch.
12- Silybum marianum (L.) Gaertn.
13- Rosa damascena Mill.
14- Glycyrrhiza glabra L.
15- Anethum graveolens L.
16- Foeniculum vulgare Mill.
17- Cichorium intybus L.
18- Nigella sativa L.
19- Hordeum vulgare L.

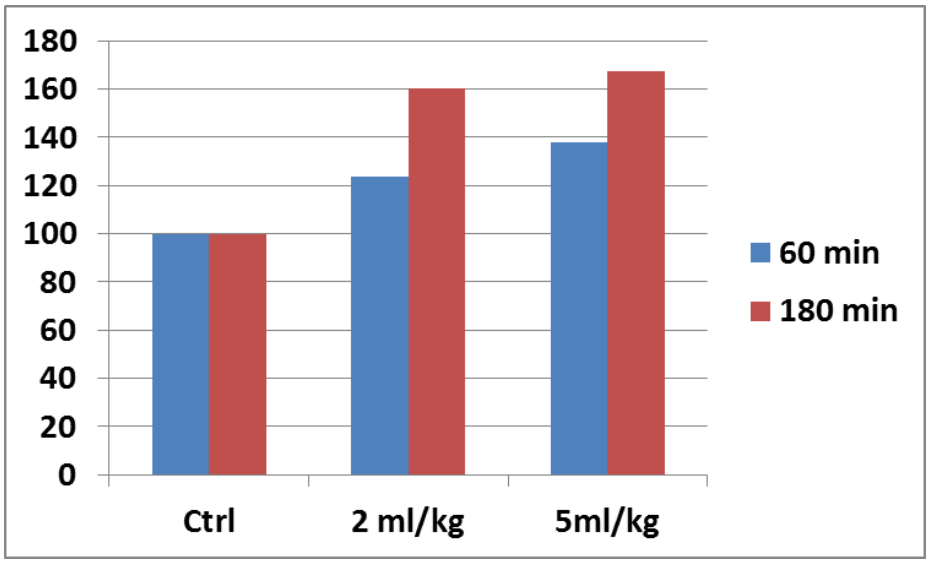

Figure-1 : Oral glucose tolerance test (GT) were compared with non-treated animals

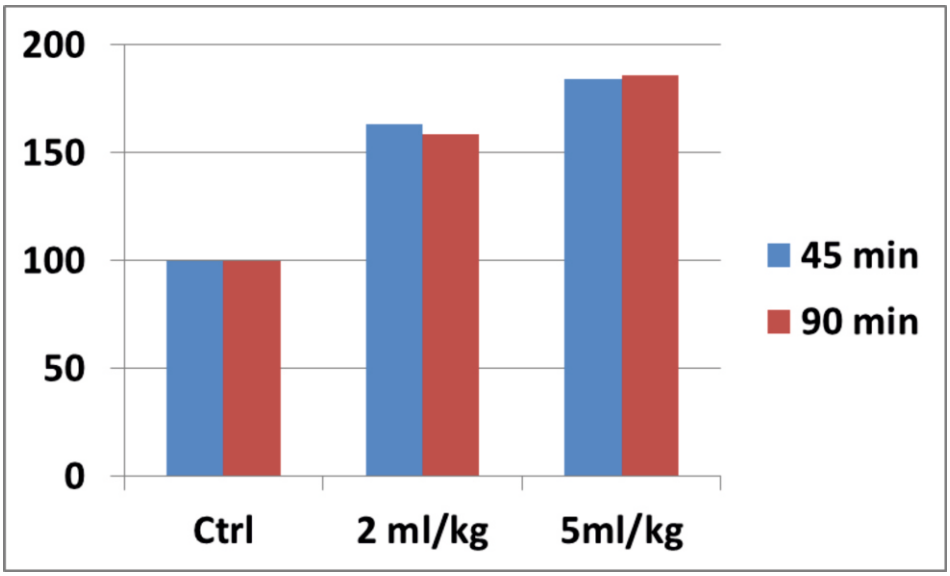

Figure-2 : insulin tolerance test (IT) in experimental rabbits were compared with non-treated animals.

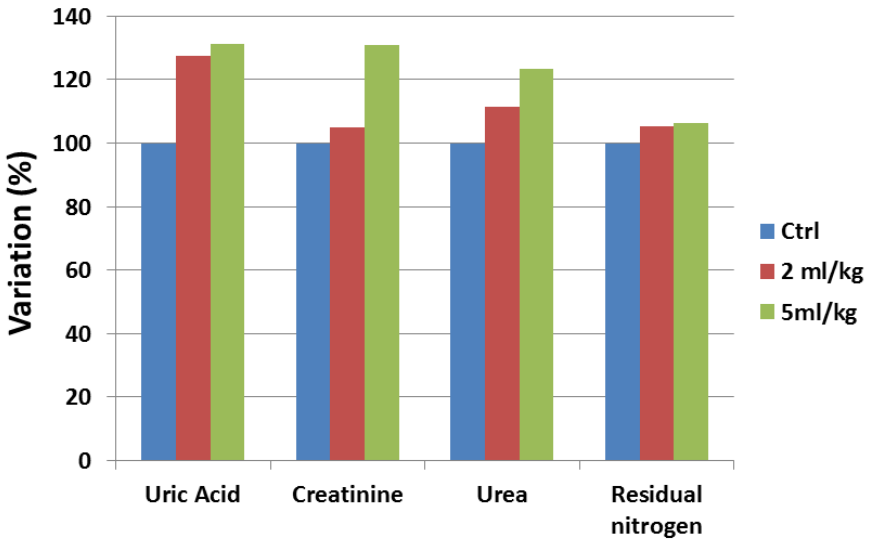

Figure-3: The renal care modification in the experimental rabbits.