Central Asian Journal of Medicine

THE POSSIBLE ROLE OF CHRONIC CONSUMPTION OF SODIUM CYCLAMAT IN THE DEVELOPMENT OF HYPERGLYCEMIA AND INSULIN RESISTANCE

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ABSTRACT

In the experiment, 10 mg/kg of sodium cyclamate which is widely used in the food industry, was dissolved in distilled water was administered to the rats during the 60 days and carbohydrate, protein, fat and microelement's metabolism were analyzed. The possible role of sodium cyclamate in the origin of insulin resistance was evaluated by chronic administration of a sodium cyclamate. The results show that it can lead to progressive increase in blood glucose levels, hyperinsulinemia and insulin resistance if consumed chronically. In addition, it was found that cyclamate affects the metabolism of proteins, fats and microelements, causing an increase in the indicators of uric acid and creatinine, lipid spectrum, and a decrease in the amount of microelements in the blood. Despite the fact that cyclamate is 30 times sweeter than sugar and economically cheaper, its permitted dose can have a harmful effect. The results suggest that the use of cyclamate should be limited or used in combination with agents that reduce its negative effects.

Key words: insulin resistance, sodium cyclamate, HOMA-IR, IRI, glycated haemoglobin, obesity, insulin, HDL, LDL, cholesterole, triglycerides.

INTRODUCTION

Sweet taste agents affect glucose control by reducing glucose reabsorption. Under the influence of these substances, impaired glucose absorption and transport in the intestines, insulin resistance, and glucose control disorders develop by

reducing the volume of insulin secretion. Systematic reviews, meta-analyses confirm that there is a connection between the consumption of sweeteners and the level of glucose or type 2 diabetes. The intestines play a big role in this, and artificial sugar substitutes affect the intestinal microflora and its function, change glucose absorption and glucose levels. The main part of glucose is absorbed through the enterocytes of the small intestine with the help of GLUT-1 located in the apical membranes, and with the help of GLUT-2 located in the basolateral membranes. Glucose binding to sweet taste receptors stimulates the secretion of GLP-1, GLP-2 and gastrointestinal peptide, and as a result, the release of GLUT-2 increases. Experiments conducted in mice revealed activation of GLUT-2 under the influence of sucralose, saccharin, and acesulfame-K. However, these substances do not affect T1R3 or α-glucose [6,16,17]. Also, sucralose, saccharin and acesulfame-K have been found to activate GLUT-2 receptors and, as a result, increase glucose reabsorption in the intestine[10,17]. Sugar and natural sugar substitutes first increase incretin synthesis. Incretin increases insulin secretion in β-cells. Artificial sugar substitutes do not affect incretin synthesis. In addition, insulin enhances binding of sugar and artificial sugar substitutes to sweet taste receptors in β-cells of the pancreas through Ca^{+2} channels and s-AMF. It was found that sugar substitutes have less effect on insulin secretion than sugar [11,16,17,19,25].

Sodium cyclamate is a product that replaces artificial sugar and has been widely used since the 50s of the last century. This substance is widely used in the food industry because it is calorie-free and resistant to high temperatures. There are several conflicting opinions on the effect of sodium cyclamate on the human body. Sodium cyclamate is mainly used in the process of making carbonated drinks, fruit juices, syrups, chewing gum, and jams. In experiments conducted on rats, the development of gall bladder cancer was determined under the influence of cyclamate. After that, this substance was removed from the list of "Completely safe" of the USA and its use in the food industry was prohibited [5]. However, cyclamate is widely used by Asian and European countries, as well as Uzbekistan. The substance cyclohexamine, which is formed as a result of the breakdown of cyclamate by the intestinal flora in the small intestine, has carcinogenic properties and has been found to cause cancer in the gall bladder and kidneys in rats [7,8,23]. The results of human experiments suggest that cyclamate may cause infertility, tachycardia, and hypertension [23]. When studying the effect of cyclamate on bone tissue, osteoblast cell cultures were used. It was found that cyclamate in the amount of 0.06 μM can have a harmful effect on the microfilaments and microtubules of osteoblasts and can reduce their mineralization and the level of Ca

ions. Cyclamate can inhibit the differentiation and proliferation of osteoblasts in in vitro cell cultures [3,5,14]. In addition, cyclamate consumption has been found to cause various pathologies in the cardiovascular and nervous system, thyroid gland adenoma, erythrocytes, leukocytes, bone cells and reproductive cells [21]. In experiments conducted on rats, consumption of drinks with 0.5-2% cyclamate significantly increased mortality [18]. Consumption of 2% cyclamate-preserved beverages caused an increase in glutamic pyruvate transaminase and lactic dehydrogenase [16]. Experiments carried out in rats for 2 months caused an increase in the amount of glucose in the blood [12,13]. Despite this, the use of cyclamate is allowed in 55 countries of the world [23].

In the literature, when sodium cyclamate was administered to rats at a dose of 10 mg/kg/day for 2 weeks, it was found that blood glucose increased by 49% [26]. The main reason for this increase is the reduction of Akkermansia muciniphila bacteria under the influence of cyclamate, which reduces the amount of lipopolysaccharides and covers the surface of the lungs with gram-negative bacteria, thickens the mucous layer, and increases the absorption of glucose. Lipopolysaccharides bind to Toll-like receptor-4 (TLR-4) in macrophages and produce inflammatory mediators. These mediators have been found to cause inflammation of pancreatic β-cells and cause insulin resistance [22,26]. Experiments conducted by Setiady et al. in rats for 5 weeks revealed that cyclamate consumption in the amount of 13.5 mg/kg/day increased blood glucose by 24.8% [22]. The above experiments show that cyclamate causes the development of insulin resistance by causing dysbiosis in the digestive tract. Due to the many conflicting opinions on the effects of sodium cyclamate, due to its wide use in various food industries, it is difficult to determine the amount of daily consumption and to evaluate its effects, the effect of its permissible daily dose has been determined. learning is becoming more important. Knowing the effect of the daily permissible dose, we will be able to assess the importance of its consumption and the level of use of nutrients that preserve it.

Materials and methods. Currently, to study the effect of sodium cyclamate, which is one of the widely used sugar substitutes in the food industry of Uzbekistan, on carbohydrate metabolism, 20 white, male, weighing 180 - Experimental rats weighing around 200 g were selected and waited for 1 week to adapt to each other. Experimental animals were kept in the vivarium of the National Reference Laboratory of Toxicology of the Sanitary-Epidemiological Peace and Public Health Committee, and sodium cyclamate was administered orally for 60 days. The experimental animals were kept at a room temperature of 22±3°C, relative humidity of 30-70%, 12 hours of light and 12 hours of darkness.

The storage and feeding of rats was carried out according to the requirements of GOST 33215-201419 [9].

The research protocol was approved by the Ethics Committee under the Ministry of Health of Republic Uzbekistan. May 19, 2022. No. 4/18-1666. 20.05.22.

For research cyclamate manufactured in LLC «НоваПродукт АГ» 108828, Moscow, Russia was purchased from "Korzinka" stores belonging to Angeley food LLC located in Tashkent. Sodium cyclamate dissolved in water in the amount of 10 mg/kg was administered orally to the experimental animals for 2 months.

Glucose tolerance test, insulin, albumin, total protein, glucose, glycated hemoglobin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), uric acid, creatinine, cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium (Ca^{2+}), sodium (Na^{+}) and potassium (K^{+}) were measured in these experimental animals before administration of sodium cyclamate as control (initial), 30 and 60 days after administration.

For biochemical and immunoenzymatic analyses, blood was taken from the tail vein of the experimental animals. For this, the rats were placed in a special metal cage corresponding to their body length, the animals' tails remained outside. In order to create a state of hyperemia, the tail of the animal was placed in hot water with a temperature of 40-50 ℃ for several minutes. The tail was then dried, a G–24 needle was inserted into the tail vein, and the collected blood was collected in a yellow tube with gel for qualitative isolation of serum. Blood was taken from the animals on the 0-, 30- and 60-days of the experiment. The blood of the experimental animals was centrifuged in a TDZ4-WS centrifuge (Hunan Xiang Yi Laboratory Instrument Development Co.,Ltd, China) at a speed of 3000 rpm for 5 minutes, and the shaped elements were separated from the blood plasma. Hemolytic blood samples were not tested.

Before starting the experiment, on the 30th day and on the 60th day of the experiment, a glucose tolerance test was performed in rats. For this purpose, the experimental animals were starved at night, after blood collection in the morning, glucose solution was injected into the stomach through an atraumatic probe at a dose of 2 g/kg. Then after 30, 60, 90 and 120 minutes, blood was taken from the animals and the amount of glucose was checked.

Biochemical analyzes of blood: albumin, total protein, glucose, glycated hemoglobin, ALAT, ASAT, uric acid, creatinine, cholesterol, triglyceride, LDL, HDL, Ca^{2+}, Na^{+} va K^{+} amounts were measured on a Humastar 100 automatic analyzer from Human Company (Germany) was determined using Human

Company's reagents. The amount of insulin was determined by the Mindray 96 semi-automatic immunoessay analyzer manufactured in the Republic of China using the Rat Elise kit for Insulin reagent belonging to ELK Biotechnology CO, LTD, USA. All the above-mentioned indicators were conducted at the Scientific Research Laboratory of the Tashkent Pediatric Medical Institute.

Based on the obtained numbers homeostasis model assessment of insulin resistance (HOMA-IR) calculated according to D.R. Matthews et al. and insulin resistance index (IRI) calculated according to M.H. Duncan et al.

In the study, statistical analyzes were carried out using JMP statistical software. The significance of differences was determined using One way ANOVA and Nonparametric Comparisons For Each Pair Using Wilcoxon Method.

Results and discussion. To evaluate the effect of sodium cyclamate on carbohydrate metabolism, a glucose tolerance test was first performed. To evaluate the glucose tolerance test in experimental animals, 2 g/kg of glucose was dissolved in water and administered to each rat through a gavage. The amount of glucose in the blood was determined before the introduction of glucose (minute 0), after 30, 60, 90 and 120 minutes after the introduction of glucose. According to the results, it was found that before sodium cyclamate administration, the glucose level in all rats was normal, and the level of glucose absorption was in good condition (Table 1). During the 30-day consumption of sodium cyclamate, the level of glucose in 0-30-60-90-120 minutes in the glucose tolerance test was respectively 65,8%, 65,7%, 67%, 68,9% and 72,2%, we can see that these increases cause blood glucose to be maintained. In the glucose tolerance test conducted on the 60th day of the experiment, glucose levels increased than initial glucose tolerant test to 73,8% at 0 minutes, to 74,4% at 30 minutes, to 71,4% at 60 minutes, to 69,4% at 90 minutes, to 75,2% at 120th minute. The results show that chronic consumption of cyclamate has a significant negative effect on carbohydrate metabolism, reducing glucose uptake. In a number of previous studies, cyclamate contained in soft drinks has an effect on carbohydrate metabolism and increases the amount of glucose in the blood, confirming the results of our experiment. [7,15,23].

Table 1.

Glucose tolerance test in experimental animals (glucose, mmol/l, M±m)

	0 minute	**30 minute**	**60 minute**	**90 minute**	**120 minute**
Initial (n-20)	3,98±0,23	5,67±0,15	5,28±0,16	4,90±0,18	4,28±0,12
30 days (n-20)	6,60±0,17	9.40±0,24	8,82±0,22	8,28±0,21	7,37±0,18
60 days (n-13*)	6,92±0,11	9,89±0,16	9,05±0,19	8,30±0,15	7,50±0,16

* At the end of the experiment, 13 rats survived

Like all sugar substitutes, sodium cyclamate consumption is thought to lead to weight gain. However, sodium cyclamate has zero calories and does not provide

energy at all [4]. Despite this, cyclamate is mainly used to enhance the effects of other sugar substitutes and may influence body weight gain by acting through taste receptors [20]. In our experiment, sodium cyclamate alone did not affect body weight gain (Table 2). On the contrary, at the end of the experiment, we can see that the body weight of the rats decreased by 7%. As a result of chronic consumption of cyclamate, it was found that the amount of glucose in the blood increased. Before the administration of cyclamate, the amount of glucose in the blood was equal to 4.4 mmol/l, on the 30th day of the experiment, it increased to 7.1 mmol/l, and on the 60th day, it was found that it increased to 7.5 mmol/l. The results show that chronic consumption of cyclamate increased blood glucose by 62.3% in the middle of the experiment, and by 69.5% at the end of the experiment. In addition, it was found that the amount of glycated hemoglobin increased by 70.3% on the 30th day of the experiment, and by 76% on the 60th day (Table 2). These increases prove that cyclamate affects blood glucose levels.

Table 2.

Effect of sodium cyclamate on blood carbohydrate metabolism indicators

	Body weight, gr	**Glucose, mmol/l**	**HbA1c %**	**Insulin, mU/l**	**HOMA-IR**	**IRI**
Initial (n-20)	175,8±4,00	4,40±0,09	3,37±0,08	9,50±0,21	1,86	1,67
30th days (n-20)	171,4±4,0	7,14±0,19*	5,74±0,10*	18,67±0,10*	5,9*	5,3*
60th days (n-13)	164,3±5,77	7,46±0,13	5,93±0,07*	15,88±1,06*	5,3*	4,7*

*-p < 0,05.

The fact that cyclamate, like other sweet-tasting substances, causes insulin resistance in the body has been cited in several literatures [1,23]. In our experiment, under the influence of cyclamate, the amount of insulin increased by 96.5% compared to the initial level on the 30th day of the experiment, and by 67.1% on the 60th day. Insulin results indicate the importance of cyclamate in causing hyperinsulinemia. The HOMA-IR index representing insulin resistance was equal to 1.86 before the start of the experiment, which indicates that the animals do not have insulin resistance. But on the 30th day of the experiment, this index was equal to 5.9, which is 3 times higher than the initial indicator, and on the 60th day, we can see that it increased by 2.8 times. The HOMA-IR index is normally up to 3, and more than 3 is a state of insulin resistance. The index of insulin resistance under the influence of sodium cyclamate was 1.67 before the start of the experiment, 5.3 on the 30th day, and 4.7 on the 60th day. HOMA-IR and IRI indicators indicate that sodium cyclamate induced insulin resistance. The general results show that the consumption of sodium cyclamate has a negative effect on carbohydrate metabolism, hyperglycemia, hyperinsulinemia, insulin resistance and, as a result, the development of type 2 diabetes. Based on these, it is

recommended to ban the use of sodium cyclamate or to replace it with other harmless natural substances.

Chronic consumption of sodium cyclamate had a significant effect on blood biochemical indicators (Table 3). Analyzing protein and nitrogen-fixing compounds metabolism, administration of sodium cyclamate for 30 days increased total protein content by 14.5% ($p<0.05$). On the 60th day of the experiment, the amount of total protein was only 5.1% higher than the initial value. Administration of sodium cyclamate to experimental animals also led to an increase in albumin: on the 30th day of the experiment, it was 18.7% more than the initial value, and on the 60th day, it was 16.8% more.

Before the start of the experiment, the amount of uric acid in the blood of animals was equal to 66.20 ± 0.89 mmol/l, after 30 days it increased by 21.5%, and after 60 days by 44.1% ($p<0,05$). The amount of creatinine in the blood was 36.66±0.82 in the intact group, and we can see that this indicator increased by 77.7% on the 30th day of the experiment, and by 103.5% on the 60th day ($p<0,01$). In the experiments conducted by Usman and others in rats, it was found that under the influence of cyclamate, the amount of creatinine increased by 51.5% and the amount of urine increased by 30.5% in 7 weeks [24]. According to the literature, it is stated that continuous administration of sodium cyclamate can cause bladder cancer in experimental animals [2].

The effect of cyclamate on liver tissue was significant, it was found that the ALAT enzyme increased by 59.7% on the 30th day of the experiment, and by 74.5% on the 60th day. These increases were statistically reliable. The 8.0% excess of ASAT enzyme on the 30th day of the experiment was statistically reliable ($p<0.01$), and the 6.7% excess on the 60th day was unreliable ($p>0.05$). Experiments conducted in rats for 7 weeks show that under the influence of cyclamate, the ALAT enzyme increased by 79.7%, and the ASAT enzyme by 61.7% [24].

Table 3.

Effect of sodium cyclamate on biochemical parameters

Parameters	Initial indicators (n-20)	Sodium Cyclamate 10 mg/kg	
		30th day (n-20)	60th days (n-13)
Indicators of metabolism of protein and nitrogen storage compounds			
Albumin g/l	37,32±0,68	44,3±0,90	43,6±0,78
Total protein g/l	66,20±0,89	75,8±0,57*	69,6±0,71
Urea mmol/l	4,99±0,13	6,06±0,21	7,19±0,18**
Creatinine mmol/l	36,66±0,82	68,58±1,10*	78,54±2,18*
Enzymes			
ALAT U/l	55,45±2,06	85,56±1,47*	96,74±2,36*
ASAT U/l	113,4±1,9	122,5±1,7	121,0±6,4

Lipid change indicators			
Cholesterine mmol/l	1,04±0,02	1,51±0,09*	1,33±0,07
Triglitserid mmol/l	0,67±0,03	0,76±0,04	0,93±0,06
LDL mmol/l	0,22±0,05	0,24±0,01	0,34±0,03
HDL mmol/l	0,53±0,02	0,56±0,02	0,99±0,07
Indicators of mineral metabolism			
Na mmol/l	143,7±1,0	151,3±1,36	133,9±1,9
K mmol/l	5,08±0,13	2,93±0,21*	2,81±0,15*
Ca mmol/l	2,09±0,01	0,86±0,02^	0,79±0,02*

*-p < 0,05.

According to the results, the amount of cholesterol and triglyceride during the experiment was equal to 1.04±0.02 and 0.67±0.03 mmol/l before the start of the experiment, and on the 30th day of the experiment, it was 45.1 and 13, respectively. We can see that it has increased by 9% and in 60 days by 27.8 and 38.2%. In scientific literature, it is stated that under the influence of cyclamate, total cholesterol and triglyceride content increased by 55.4% and 49.4% [24]. These indicators indicate that sodium cyclamate has a negative effect on fat metabolism. The administration of sodium cyclamate caused an increase in the amount of LDL and HDL by 54.5 and 86.8%, respectively, on the 60th day of the experiment, without affecting the amount of LDL and HDL in the blood of experimental animals on the 30th day of the experiment. Unlike ours, Usman et al.'s experimental results showed that LDL increased by 66.7% and HDL decreased by 12.7% [24].

In addition, the chronic consumption of sodium cyclamate affects the metabolism of microelements, and on the 30th day of the experiment, the amount of Na^+ increases by 5.3%, and the amount of K^+ and Ca^{2+} decreases by 43.4% and 59%, respectively. At the end of the experiment was found to cause a decrease in the amounts of Na^+, K+ and Ca^{2+} by 6.8, 45.7 and 63.4%, respectively. The level of microelements may be reduced due to the development of dysbacteriosis in the intestines caused by the influence of sodium cyclamate, due to the violation of the absorption of sodium, calcium and potassium microelements.

Conclusion. It has been shown that sodium cyclamate 10 mg/kg daily dose of sodium cyclamate, a substance that gives a sweet taste over time, has an effect on carbohydrate metabolism in the body and can produce hyperchemistry and insulin resistance in the blood if it is taken for a long time. , sodium cyclamate has a damaging effect on the processing function, causing a dangerous and sharp increase in the amount of creatinine in the blood. The effect of cyclamate on the liver tissue is also noticeable, causing an increase in the amount of the enzyme alanine aminotransferase. It was found that cyclamate causes a progressive increase in fat production indicators in experimental animals until the end of the experiment. The effect of sodium cyclamate on the intestinal microflora can be

attributed to the purification of absorption of microelements. Scientific research shows the health effects of cyclamate in food production, type 2 diabetes, and the need to load sodium cyclamate in food production or make it safe for other health concerns.

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