The Comparison of the Protective Effect of L-Carnitine, Chromium and Vitamin D with Metformin on Liver, Antioxidant Enzyme Activity, and Iron Metabolism in Diabetic Rats

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Received: 18 January 2021

Accepted: 14 April 2021

Published in May 2021

Abstract

Objective: Due to the lack of information about the positive or negative effects of L-carnitine, chromium, vitamin D, and the uptake of a safe dietary supplement to reduce the effects of diabetes, it seems essential to determine the effects of these drugs on diabetes.

Materials and Methods: Wistar rats were divided into 12 groups, control, diabetic, and intact, each of which was treated with metformin, L-carnitine, vitamin D, and chromium or a combination of L-carnitine, vitamin D, and chromium. The serum levels of liver function parameters, iron, TIBC, Catalase and GPX activities were measured. Data were statistically analyzed by one-way ANOVA using SPSS 18 software. Statistical significance among the groups was determined using LSD test, and *P*-value< 0.05 was considered significant in all cases.

Results: AST activity in diabetic groups and those receiving chromium and L-carnitine has significantly reduced (*P*-value= 0.009). A significant reduction in ALT and ALP activity in the diabetic groups receiving vitamin D and combined and non-diabetic groups receiving metformin were observed, in contrast to a significant increase in ALT activity in diabetic recipients of L-carnitine (*P*-value= 0.009).

Conclusion: L-carnitine, chromium, and vitamin D supplements have synergistic effects and a combination of them has the best protective effect on factors that have been studied.

Keywords: Diabetes mellitus, L-carnitine, Chromium, Vitamin D, Metformin

Introduction

Diabetes mellitus is a metabolic syndrome, cardiovascular, and interconnected nervous. This metabolic syndrome occurs due to the lack of or deficiency in insulin secretion or ineffective insulin activity. In course of the disease, some free active radicals are produced that can provoke the cytotoxic destruction of β-cells and inducing Type 1 diabetes mellitus (DM). During the last decades, non-bone diseases associated with vitamin D deficiency (like diabetes) have been known and brought us to

[DOI: 10.18502/ijdo.v13i2.6476]

the conclusion that vitamin D deficiency and diabetes have common risk factors such as obesity and ageing, (1). Metformin, a hypoglycemic drug, has antioxidant effects associated with increasing of glutathione and reducing of lipid peroxidation in DM (2).

L-carnitine increases the catabolism of glucose by increasing the activity of pyruvate dehydrogenase. In addition, some studies have shown low levels of L-carnitine in serum of diabetic patients, therefore the consumption of L-carnitine is effective in these patients in order to improve the catabolism of glucose and chromium is another essential nutrient for metabolism of sugars and fats. An improvement of 40 to 50 percent in impaired glucose tolerance and also in serum cholesterol of diabetic patients was detected after taking chromium supplement (3).

Decreasing oxidants and increasing antioxidants can result in the reduction of oxidative stress and lipid peroxidation markers in diabetic patients. Catalase is an unusual enzyme because although hydrogen peroxide is its only substrate, it follows the Ping Pong reaction mechanism. First, its cofactor is oxidized by hydrogen peroxide, followed by transferring limited oxygen into the second molecule resulting in the reconstruction of the substrate (4).

Glutathione Peroxidase catalyzes hydrogen peroxide and some other organic hydroperoxides, using glutathione as the reducing substance. Therefore, changing the statues of antioxidant defense systems increases oxidative stress in DM (5). Due to the injury and death of liver cells, their intracellular enzymes move into the blood, so their activity can be measured. ALT, AST and ALP are some of the most important ones of these enzymes (6).

Due to the lack of information about the protective effect of L-carnitine, chromium, and vitamin D, compared to metformin on liver activity, iron metabolism, and catalase and glutathione peroxidase activity in DM, it seems essential to determine the effects of these drugs on Streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

In this experimental study, sixty male Wistar rats with an average weight of 250-300 g in standard conditions were obtained from Islamic Azad University of Falavarjan. After adaptation to the environment in standard conditions they were randomly subdivided into 12 groups of 5 rats as follows: 6 groups including control group, and metformin, L-carnitine, chromium, and a combination of L-carnitine, chromium and vitamin D (combined group) receivers were injected intraperitoneally with 60 mg/kg of streptozotocin (STZ) (Sigma, Germany) per rat, to induce DM. Other groups were intact while receiving the same supplements as the first six groups.

STZ impairs pancreatic cells and reduce insulin production as a result. Blood sugar with blood obtained from the animal's tail was measured using a Bionime GM 110 (Taiwan) glucometer and glucose strips, before, as well as 24 hours, 72 hours, and one week after STZ injection. Rats with blood glucose levels more than 300 mg/dl were considered as DM. L-carnitine (200 mg/kg), chromium (2 mg/kg), and metformin (2 mg/kg) were injected intraperitoneally, and Vitamin D (0.06 µg) in almond oil was fed orally (via gavage) to groups daily, for 30 days, at certain hour. After 30 days, blood serum was used to measure glucose level, liver function parameters (AST, ALT, ALP), iron, TIBC, and catalase and glutathione peroxidase enzyme activity. Data were statistically analyzed by one-way ANOVA using SPSS 18 software. Statistical significance among the groups was determined using LSD test and P-value<0.05 was considered significant in all cases.

Ethical considerations

This research proposal was approved in Islamic Azad University of Falavarjan (Ethics approved and thesis code: 17230520941013)

[DOI: 10.18502/ijdo.v13i2.6476]

Results

Table 1 presents the results of hepatic factors levels. The AST activity in non-diabetic group treated with chromium and L-carnitine compared to diabetic control group was significantly decreased (*P*-value= 0.009). In non-diabetic metformin receivers, a significant reduction of the ALT activity was observed. However, in diabetic groups receiving metformin and L-carnitine, a significant increase in the ALT activity was detected. In contrast, the ALT activity of the diabetic groups receiving vitamin D and combined supplements has notably reduced (*P*-value= 0.009). The activity of ALP has significantly decreased in both metformin receiver groups, and diabetic combined group (*P*-value= 0.008) The serum levels of iron and TIBC in non-diabetic and diabetic groups is presented in Table 2. Statistical comparison of serum levels (µg/dl), revealed that the mean serum level of TIBC and Fe (µg/dl) were not significantly different from each other in non-diabetic groups (*P*-value>0.05), whereas a considerable increase was monitored in TIBC amounts in diabetic group receiving a combined group of L-carnitine, chromium, and vitamin D (*P*-value= 0.027), and in iron levels in diabetic groups taking metformin, L-carnitine, vitamin D and combined supplements (*P*-value= 0.015).

According to figure 1 the activity of glutathione peroxidase (IU/L), is lower in all non-diabetic groups, compared to the diabetic groups, except for the control group, where the enzyme activity of non-diabetic group is higher than that of the diabetic group (*P*-value= 0.035). The GPX activity of diabetic rates taking chromium was significantly higher

Table 1. Liver factors measured in diabetic and non-diabetic groups

Group		AST(IU/L)	*P*-value@	ALT(IU/L)	*P*-value@	ALP (IU/L)	*P*-value@
Control	Non Diabetic	186.6 (± 34.58)	>0.05	62.60 (± 13.51)	0.0001	672.6 (± 120.5)	0.0001
	Diabetic	276.6 (± 49.5)		167.96 (± 25.12)		4520.8 (± 397.56)	
Metformin	Non Diabetic	161.6 (± 28.16)	>0.05	47.90 (± 8.15)	0.0001	385.4 (± 129.2)	0.0001
	Diabetic	149.8 (± 31.35)		245.2 (± 51.23)		3231.8 (± 1677.93)	
Vitamin D	Non Diabetic	165.6 (± 29.29)	0.0001	71.10 (± 10.57)	0.0001	737.4 (± 253.4)	0.0001
	Diabetic	105.31 (± 2.73)		107.12 (± 20.86)		3634.2 (± 973.24)	
Chromium	Non Diabetic	145.4 (± 13.59)	0.0001	59.40 (± 5.31)	0.0001	801.2 (± 235.4)	0.0001
	Diabetic	121.32 (± 32.03)		216.6 (± 48.38)		4545.2 (± 158.79)	
L-Carnitine	Non Diabetic	151 (± 19.91)	0.0001	53.40 (± 3.36)	0.0001	467.2 (± 46.69)	0.0001
	Diabetic	157.8 (± 13.66)		273.2 (± 49.35)		4682.8 (± 251.72)	
L+C+D	Non Diabetic	202 (± 9.53)	0.0001	59.30 (± 8.25)	0.0001	674.2 (± 191.7)	0.0001
	Diabetic	141.4 (± 36.47)		48.1 (± 27.42)		2167.6 (± 734.53)	
P*-value*	Non Diabetic	0.009		0.009		0.008	
	Diabetic	0.014		0.00		0.00	

P-value**: Separated between diabetic and non-diabetic groups
P-value@: Any factor between diabetic and non-diabetic groups

Table 2. Fe and TIBC measured in diabetic and non-diabetic groups

Group		Fe	*P*-value@	TIBC	*P*-value@
Control	Non Diabetic	114.2 (± 16.76)	>0.05	284 (± 23.42)	>0.05
	Diabetic	102.75 (± 18.25)		290.36 (± 1.78)	
Metformin	Non Diabetic	131.8 (± 15.59)	0.042	285.6 (± 19.70)	>0.05
	Diabetic	121.7 (± 53.57)		302 (± 11.2)	
Vitamin D	Non Diabetic	166.8 (± 19.17)	0.024	304.6 ± 23.42	>0.05
	Diabetic	191.7 (± 0.84)		310 (± 17.63)	
Chromium	Non Diabetic	142.2 (± 20.19)	0.035	320 (± 52.75)	>0.05
	Diabetic	98.5 (± 24.82)		292.2 (± 6.68)	
L-Carnitine	Non Diabetic	150 (± 14.67)	>0.05	287.8 (± 17.45)	>0.05
	Diabetic	143.1 (± 36.93)		290.2 (± 10.98)	
L+C+D	Non Diabetic	180.6 (± 3.91)	0.012	292.2 (± 1.9)	0.015
	Diabetic	210.7 (± 57.97)		324.4 (± 28.78)	
P*-value*	Non Diabetic	>0.05		>0.05	
	Diabetic	0.015		0.027	

P-value**: Separated between diabetic and non-diabetic groups
P-value@: Any factor between diabetic and non-diabetic groups

[DOI: 10.18502/ijdo.v13i2.6476]

than that of the non-diabetic group, which showed the least enzyme activity among all groups (*P*-value= 0.0001). Based on the results demonstrated in Figure 2, the average enzyme activity of catalase showed a remarkable improvement in all diabetic groups receiving supplement, compared to the diabetic control group (*P*-value= 0.0001).

Discussion

The production of free radicals and the alteration in the activity of antioxidant enzymes play a crucial role in the incidence of diabetic complications and the development of insulin resistance in these patients. The more we know about the activity of antioxidant enzymes, and the factors affecting them, in diabetic patients; the more fruitful and promising our pharmaceutical and nutritional interventions to reduce oxidative stress in type 1 diabetic patients, will be (7,8).

Studies on diabetic rats revealed that STZ had damaging and toxic effects not only on pancreatic β-cells, but also on other organs, including liver, leading to a reduction in the amount of hemoglobin, total protein, and albumin in STZ-induced diabetic rats (9,10). A study on the protective effect of vitamin D and umbelliferon on STZ-induced diabetic mice

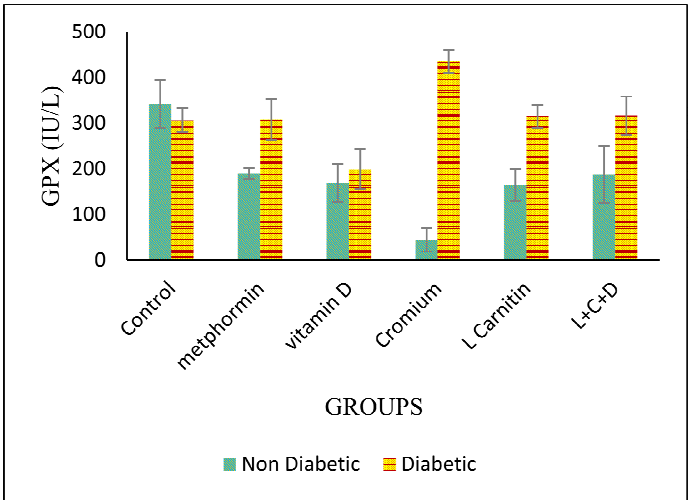

Figure 1. The mean glutathione peroxidase enzymatic activity in non-diabetic and diabetic groups (*P*-value= 0.0001)

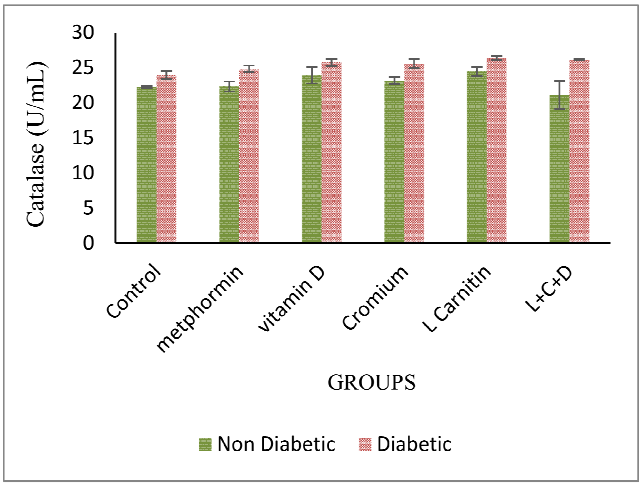

Figure 2. The average activity of catalase enzyme in non-diabetic and diabetic groups (*P*-value =0.0001)

[DOI: 10.18502/ijdo.v13i2.6476]

indicated a decrease in AST enzyme activity, which is in agreement with our results (11).

Lack of vitamin D influences the postprandial blood glucose and insulin response; therefore, vitamin D supplements may optimize this process. Metformin improves hyperglycemia by reducing the intestinal absorption of glucose and glucose production, by preventing gluconeogenesis, and enhancing peripheral insulin sensitivity. The synergistic effect of vitamin D and metformin seems to improve the levels of liver enzymes (12) which is in accordance with the obtained results of vitamin D consumption in the present study. On a study in 2013, examining the effect of metformin on the oxidative stress responses in diabetic rats, a significant decline in the activity of catalase and glutathione peroxidase was observed in diabetic rats, compared to the non-diabetic control group (13). The same result was obtained in the present study while examining the effect of metformin on the activity of catalase and glutathione peroxidase on diabetic rats. Significant changes in the activity of catalase and glutathione peroxidase in diabetic group, compared to the non-diabetic group, indicates the damaging effect of diabetes-related oxidative responses on liver. In our study, the consumption of metformin inhibited the decline in the level of serum biochemical parameters.

The evaluation of the effect of chromium chloride on glucose level and hemoglobin in diabetic patients demonstrated a sharp decrease of blood glucose (14). It is suggested that chromium improves insulin binding and rises the number of insulin receptors, and enhances β-cells and overall insulin sensitivity (15). In our research, a certain dose of chromium chloride has significantly reduced the level of liver enzyme AST.

L-carnitine plays a great role in fatty acid metabolism, therefore, a decrease in carnitine increases fatty acids in the blood, followed by increased insulin resistance paving the way for diabetes. Also, based on the metabolic pathways of L-carnitine, it probably plays a protective role by increasing stored iron and decreasing free iron (16). Thus, diabetic patients especially those with chronic complications of diabetes will have a greater need for L-carnitine.

In this study, all diabetic groups compared to non-diabetic groups showed a significant decrease in the activity of liver enzymes. ALT activity had the greatest reduction in the diabetic group receiving combination therapy of L-carnitine, chromium, and vitamin D, followed by the vitamin D receivers who showed the remarkable and meaningful reduced in the activity of this enzyme.

According to the results of the present study, metformin can be recommended as an acceptable drug, possessing hypoglycemic, anti-lipidemic and antioxidant properties. Therefore, its prescription may be beneficial for obese diabetic patients. The study of other hypoglycemic drugs, used by diabetic patients, and their synergistic effects, while used alongside metformin, is suggested for future directions.

Due to the lack of prominent research on type I diabetes in Iran, it would be of great value to investigate the protective effect of other supplements, such as other vitamins or the synergistic effect of vitamin D with herbal medicines.

Conclusions

According to the results, it was found that all tested treatments were fairly effective on parameters that had been examined. But the group receiving vitamin D, among all other groups, had more effective and significantly different results from the rest. In the group receiving the combination of L-carnitine, chromium, and vitamin D more effective and positive impacts were also detected. The obtained results not only proved the protective roles of L-carnitine, chromium, and vitamin D but also showed that each of these agents played its protective role in a different way. In terms of pharmaceutical combination, Metformin seemed appropriate which had the hypoglycemic, co-antilipidemic, and co-antioxidant effects.

[DOI: 10.18502/ijdo.v13i2.6476]

Acknowledgments
The authors are grateful to the Islamic Azad University, Falavarjan branch for their cooperation and providing necessary facilities.

Funding
No funding.

Conflict of Interest
There are no conflicts of interest.

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[DOI: 10.18502/ijdo.v13i2.6476]