A CONTRIBUIÇÃO DO ZINCO NO TRATAMENTO DA DIABETES

O orientador,

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Contents

ABSTRACT

Due to, in part, to the expansion of western culture and fast and uncontrolled urbanization, diabetes is, today, a particularly present and onerous disease for world's healthcare systems. Besides, WHO foresees the incidence tax of this disease will reach almost the double of cases in 15/20 year time.

In this review it is done a brief reference to the most frequent complications of diabetes and the pharmacological treatments available today and, most importantly, the role of zinc in diabetes and mainly its potential use as an antidiabetic agent.

They were analyzed for this several articles in which was made the connection between zinc levels in different compartments of the human body and the complications usually associated with diabetes. With more or less strength, they suggested a link between zinc levels and the incidence of different complications.

They were also analyzed studies using a multiplicity of medicines involving zinc as an antidiabetic agent. In the majority of the cases, especially in the studies that provided the subjects with oral zinc nanoparticles, the result were promising

Keywords: diabetes mellitus, zinc, diabetes treatment, nanoparticles, nanotechnology

RESUMO

Devido, em parte, à expansão da cultura ocidental e da urbanização rápida e descontrolada, a diabetes é hoje uma doença particularmente presente e onerosa para os sistemas de saúde mundiais. Além disso, a WHO prevê que a taxa de incidência desta cresça quase para o dobro no espaço de 15/20 anos.

Neste trabalho pretende-se referenciar de forma breve as complicações mais frequentes dos diabéticos e os tratamentos farmacológicos já existentes usados atualmente no seu tratamento e, mais fundamentalmente, o papel do zinco na diabetes o seu possível uso como agente antidiabético.

Para isso foram analisados vários artigos em que se faz a conexão entre os níveis de zinco em diferentes compartimentos do corpo humano e várias complicações associadas à diabetes. Com maior ou menor importância, estes sugeriram uma ligação entre os níveis observados e as incidências das complicações.

Analisaram-se também estudos usando uma multiplicidade de fármacos envolvendo zinco como agente antidiabético. Na maioria dos casos, em especial nos estudos que se dispensava o zinco em nanopartículas orais, os resultados foram prometedores.

Palavras-chave: diabetes mellitus, zinco, tratamento da diabetes, nanopartículas, nanotecnologia

Introduction

World Health Organization (WHO) classifies diabetes as "a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type 2 diabetes (T2D), usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. In the past three decades the prevalence of T2D has risen dramatically in countries of all income levels.

Type 1 diabetes (T1D), once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself. For people living with diabetes, access to affordable treatment, including insulin, is critical to their survival. There is a globally agreed target to halt the rise in diabetes and obesity by 2025." [1]

The most notorious complications of diabetes are:

- High risk of developing foot ulcers and infection, which may lead to amputation
- Diabetic retinopathy as a consequence of the damage to the optical small blood vessels
- Neuropathy, either by direct action of the hyperglycemia or due to the decreased irrigation by the small blood vessels

Except for other less common types, we can consider diabetes cases as being one of these two: type1diabetes (T1D) and type 2 diabetes (T2D).

Type 1 Diabetes

Formerly known as insulin-dependent diabetes, it is usually diagnosed in childhood or adolescence (although it may appear later), and it is rarely associated with obesity (in fact, patients often present signs of weight loss). Patients with T1D can't

produce insulin at all, which will lead to severe hyperglycemia. Due to this great lack of insulin, the most severe complications of diabetes, such as ketoacidosis and coma, are seen more often in this kind of diabetics. [1]

Although the etiology can vary, it is, most of the times caused by autoimmunity against β-cells in the pancreas (Type 1A). Other cases are described as being non-autoimmune or idiopathic (type 1B). [2]

The treatment of this kind of diabetics is mainly done with administration of insulin (either short-, intermediate- or long-acting insulin). [1]

Type 2 Diabetes

This type of diabetes is associated with unhealthy diets, life habits (lack of physical exercise) and obesity. Although the majority of cases are diagnosed in adulthood, there is an increasing number of patients with this condition diagnosed in childhood or adolescence.

Opposed to the type 1, patients with T2D can produce insulin, but it is often scarce quantities. In addition, some patients produce deficient insulin or the tissues themselves present resistance to insulin action. [1]

Along with type 1 and type 2, there are several other kind of Diabetes (gestational, diabetes secondary to pancreatic disease, etc.). [1]

Diabetes is showing a global prevalence of 382 million in 2013 (expected to grow to 592 million in 2035), and type 2 diabetes representing more than 85% of the cases. The numbers show an increase in type 1 diabetes cases as well as in type 2. This increase in type 2 diabetes cases seem to be linked with rapid urbanization of formerly rural areas, increased living standards and the wide adoption of western diets. [2]

This facts make prevention (education towards the importance of healthy diets and physical exercise) and the discovery of cheaper and more effective pharmacological treatments of diabetes a priority for health organizations worldwide, especially in rapid growing countries.

Contemporary Treatments of Diabetes

Pharmacological treatment for diabetic patients is carefully planned according to the action of the disease on the body. It is important to evaluate first if the patient is insulin-dependent or insulin resistant (there might be cases where the patient shows both). [37]

The following table [3] shows the most relevant drugs acting on diabetes currently available.

Table I – Pharmacological classification and administration route of the most relevant drugs acting on diabetes.

Pharmacological Subgroup	Drug	Route
Biguanides	Metformin	Oral
Thiazolidinediones	Rosiglitazone	Oral
	Pioglitazone	
Alpha Glucosidase Inhibitors	Acarbose	Oral
	Miglitol	
Sulfonylureas	Chlorpropamide	Oral
	Glibenclamide	
	Glimepiride	
	Glipizide	
	Tolazamide	
	Tolbutamide	
Glinides	Nateglinide	Oral

	Repaglinide	
Exenatide and Extended Release Exenatide		Subcutaneous
Liraglutide		Subcutaneous
Pramlintide		Subcutaneous
Dipeptidyl Peptidase-4-Inhibitors	Sitagliptin	Oral
	Saxagliptin	
	Linagliptin	
Rapid Release Bromocriptine		Oral
SGLT-2 Inhibitors	Canagliflozin	Oral
	Dapagliflozin	

Along with these drugs, insulin and its derivatives are still the most effective method for controlling diabetes. Table II shows the onset, peak and duration of the most usual insulin forms, according to Mario Skugor [3].

Table II – Commonly Used Insulin Forms

Insulin	Onset	Peak	Duration
Rapid-Acting			
Insulin Aspart	5 to 15 mins	30 to 90 mins	< 5 hours
Insulin Lispro			
Insulin Glulisine			
Short-Acting			
Insulin	30 to 60 mins	2 to 3 hours	5 to 8 hours
Intermediate			
Neutral Protamine Hagedon	2 to 4 hours	4 to 10 hours	10 to 16 hours
Long-Acting			

<table>
<tr><td>Insulin Glargine</td><td>2 to 4 hours</td><td rowspan="2">No peak</td><td>20 to 24 hours</td></tr>
<tr><td>Insulin Detemir</td><td>3 to 8 hours</td><td>17 to 24 hours</td></tr>
<tr><td colspan="4">Pre-Mixed</td></tr>
<tr><td>75% Insulin lispro protamine
25% insulin lispro</td><td rowspan="3">5 to 15 mins</td><td rowspan="4">Dual</td><td rowspan="4">10 to 16 hours</td></tr>
<tr><td>50% Insulin lispro protamine
50% insulin lispro</td></tr>
<tr><td>70% Insulin lispro protamine
30% insulin aspart</td></tr>
<tr><td>70% NPH
30% insulin</td><td>30 to 60 mins</td></tr>
</table>

Aligned with a personalized pharmacological prescription, diabetics must put effort on maintaining a good control of their glycaemia. This can be done in two ways:

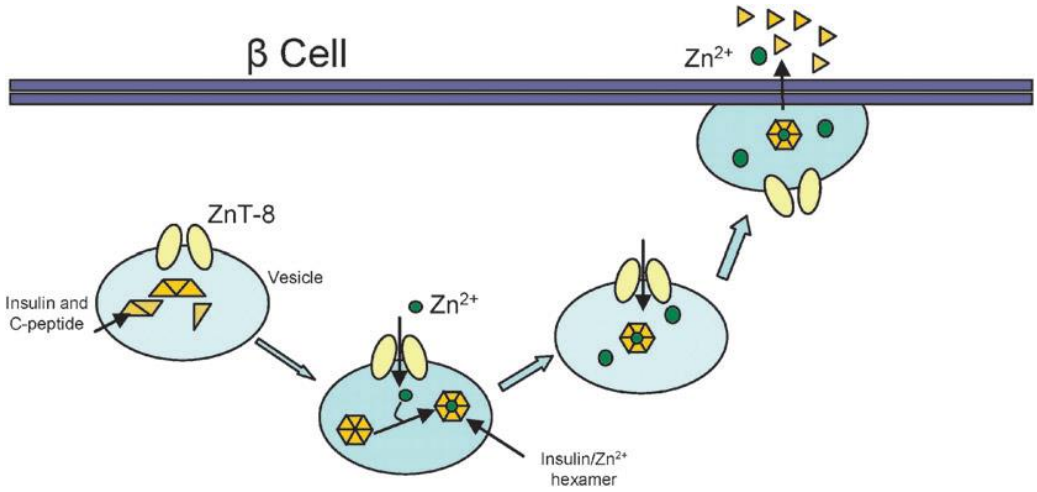
- **Self-Monitoring of Blood Glucose (SMBG):** a really common glucose measuring procedure for patients with diabetes. T1D patients are recommended to measure glycaemia at least 3 times a day. Although patients with T1D and insulin-dependent T2D seem to benefit according to some studies, the effect of SMBG on the rest of type-2 DM patients is unclear. According to American Diabetes Association (ADA), a non-diabetic individual must have his glucose levels between 70-130 mg/dL in preprandial regime and under 180 mg/dL in postprandial. [1]

- **Hemoglobin A_{1C}:** Also known as irreversibly glycosylated hemoglobin is a periodical examination that's directly related to the blood glucose levels

for the past 2 or 3 months and can be a good predictor for microvascular complications. It is recommended that all the diabetics do the test at least 2 times (if the disease is controlled). The ones with unstable glucose levels or that are initiating a new pharmaceutical regime are recommended to do the test 4 times a year. This test still has flaws, as it can be influenced by a rapid red blood-cell turnover, blood loss and anemic states, so this has to be taken into consideration when analyzing the values. The goal in terms of values are below 7% for the most cases. Patients with T1D or pregnant T2D patients are recommended to keep H A_{1C} below 6%.[1]

Zinc and Insulin Production

Zinc plays an important role in the structure and function of insulin, and such, it is no surprise that β-cells have very high zinc levels in comparison with other cells. Inside the β-cell, zinc is mostly located (around 70%) in insulin secretory granules (ISG), with a total concentration situated between 10 and 20 mM. [3, 4] The transporter ZnT8, a cation diffusion facilitator, [48] is the responsible for this high concentration. [6, 7] Being this transporter so important to the synthesis of insulin, it is expectable that its depletion may have a negative effect on this production.



1ZnT-8 role in insulin secretion (38)

We know that *Slc30a8* is the gene responsible for the synthesis of ZnT8 [39] but is difficult to determine what influences its expression, mainly because the proximal Slc30a8 promotor is only active in stable and non-transient transfections. [8] However, we do know that some factors like zinc depletion and glucose and cytokine treatments can decrease the gene's expression on mice [9, 10] (although cytokine treatments have shown little effect on the expression of the gene in human cell cultures) [11]. This regulation might contribute to disease since acute suppression of ZnT8 impairs GSIS (glucose-stimulated insulin secretion) in rodent β-cells [12] and *in vivo* may represent an early event in diabetes. [13]

In studies involving the global [6, 14, 15, 16] or β-cell-specific [17, 18] suppression on *Slc30a8* suggested minor or no changes to the appearance of the β-cell in mice. However, all of them (except [15]) saw major changes in ISG appearance and the majority could link a decrease of zinc content in the islets.

Totally unexpected was the results of 6 studies investigating the effects of *Slc30a8* suppression in GSIS: two of them showed an increase in GSIS [6, 18], two other showed no signs of correlation [15, 16] and the other two showed a decreased GSIS. All of these studies were initiated with the premise they would prove that an absence in ZnT8 (or loss of function) were directly linked with an increased risk for developing T2D.

Davidson *et al.* [19] propose this conclusions imply that the zinc concentration in ISG is not as important as previously thought, and a relatively low concentration of zinc in these granules is sufficient to support the insulin synthesis. Nevertheless, given the high zinc turnover observed in β-cells (24 hours) relative to other cells [20], can lead to stress of ZnT8 transporters in individuals with unpaired expression, which can lead to states of glucose intolerance or even T2D.

Relation between Zinc levels and Diabetes Mellitus Complications

There are various studies already indicating the relationship between serum zinc levels and microvascular problems in diabetic patients. On the other hand, these studies were often limited regarding the size of the studied population. Furthermore, most of them only focused in one microvascular complication, disregarding the fact that were probably more important independent risk factors that influence significantly these complications alongside the serum zinc levels. Therefore, failed to prove with sufficient probability the link between microvascular complications and serum zinc levels, as presented in (36).

A link between serum levels and microvascular complications in diabetic patients was suggested by Ying-Ying *et al.* [21], through a study done in Peking University People's Hospital, between the 30th of May, 2013 and the 31st of March, 2014.

In the case of diabetic nephropathy, their data even suggested serum zinc levels as being an independent risk factor. They've successfully linked albumin/creatinine ratio with serum zinc levels, showing a negative correlation between the two.

Logistic regression analysis of zinc level and diabetic microvascular complications in patients with T2D

Microvascular Complications	Odds Ratio (95% CI)	p value
Diabetic Retinopathy	0.900 (0.782–1.035)	0.138
Diabetic Nephropathy	0.869 (0.765–0.987)	0.031*
Diabetic Peripheral Neuropathy	0.967 (0.882–1.060)	0.468

*Statistical significance. *OR*: Odds ratio; 95% *CI*: Confidence interval; DR: Diabetic retinopathy; DN: Diabetic nephropathy; DPN: Diabetic peripheral neuropathy. Data by Ying-Ying *et al.* [21]

Although they've achieved to suggest this, they've still failed to make a definitive connection between diabetic retinopathy and zinc serum levels as being an independent risk factor for this complications. Nevertheless, zinc is, as proven by Miao, X. *et al* [22] as having a protective effect on the retina, via the stabilization of the membrane structure, activation of metallothionein, the clearing of free radicals and inhibition of lipid peroxidation, as well as the possibility of reducing the expression of endothelial growth factor, therefore inhibiting neovascularization and exudation.

As for diabetic peripheral neuropathy (DPN), they couldn't prove serum zinc levels as being an independent risk factor. That being said, they could prove a negative correlation between serum zinc levels and the prevalence of DPN. As oxidative stress is proven to be a major risk for developing DPN [23], and zinc activity as an antioxidant and promotor of metallothionein production [24], zinc probably has an important protective effect, and the visible reduced serum zinc levels in patients with this complication helps corroborating this idea.

A study done by Diha J. Al-Timimi *et al.* in Duhok Diabetes Center, Iraq, from April to October (2014)[25] was able to establish a connection between lower serum zinc levels and genetic predisposition for T2D. This was done by analyzing zinc levels in siblings of T2D patients.

Serum Zinc Levels of Siblings of T2D Patients vs. Healthy Individuals

Zinc Status	N.º of Siblings	N.º of Healthy Individuals	p-value
Severe Zinc Deficiency (<50 µg/dL)	0 (0,0)	0 (0,0)	
Marginal Hypozincaemia (50-70 µg/dL)	28 (13,9%)	7 (5,4%)	0,035

Normozincaemia (>70-129 µg/dL)	164 (81,6%)	120 (92,3%)	0,170
Hyperzincaemia (>129 µg/dL)	9 (4,5%)	3 (2,3%)	0,234

Diha J. Al-Timimi *et al.*[25]

On the other hand, Yary *et al.*, have presented in October 2015[26] the results for a prospective study involving 2682 eastern Finnish men proving an independent correlation between serum zinc levels and the increased risk of T2D incidence in middle-aged men. They suggest that this is due to the overstimulation of pancreatic β-cells leads to excess production of insulin, which in time can be the cause of insulin resistance, due to the exhaustion of insulin receptors.

Zinc related drugs in Diabetes Treatment

The current view towards the treatment or prevention of diabetes via zinc or zinc based medicines is of considerable skepticism. It is long known the importance of zinc in pancreatic function and diabetes [27]. However, it's been proven difficult to establish a safe and definitive link between zinc levels and insulin-production. Although not enough, there are several studies on the matter. Unfortunately, there is a vast difference regarding their inferences.

In 2002, Marreiro *et al*. conducted a study [36] involving obese women to assess the effects of zinc supplementation on insulin production and, ultimately, diabetes prevention. The results were anything but promising: zinc supplemented women didn't show any major alterations of the studied variables such as anthropometric characteristics (body mass index, skinfold thickness…), lipid metabolism (triglycerides, total cholesterol, LDL, HDL…) and plasma insulin when compared to the ones who received placebo.

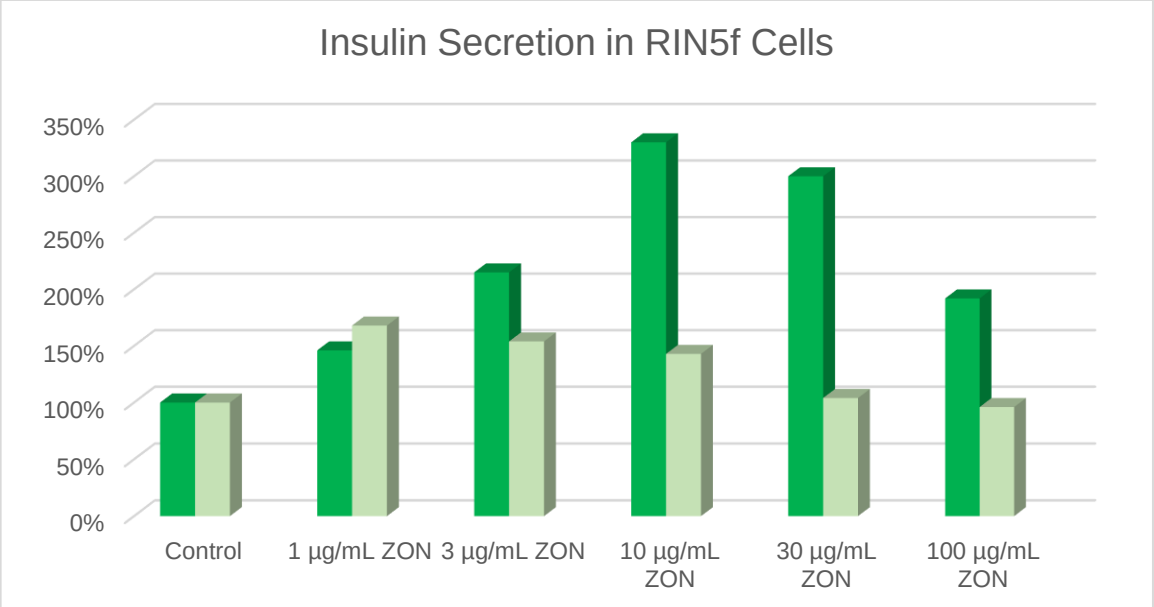
This is an example of the inefficacy of normal oral zinc supplements as diabetes prevention/treatment drugs.

Zinc Nanoparticles

Lately, the possibility of using nanotechnology to as means to best deliver the zinc are under study.

In 2012, Rinku Umrani and Kishore Paknikar, form the Agharkar Institute in India, [28] have claimed to design the first medicine against diabetes based on a zinc oxide nanoparticle (ZON). Their study was made on rat insulinoma cells (RIN5f) and Human Hepatocellular Carcinoma Cells (HepG2), as well as on Wistar Rats, diabetes-induced by sterptozotocin (for T1D 45mg/kg dose in tail vein in adults, and 90mg/kg intraperitoneally to 5-days-old rats, allowing to grow to 12 weeks for T2D).

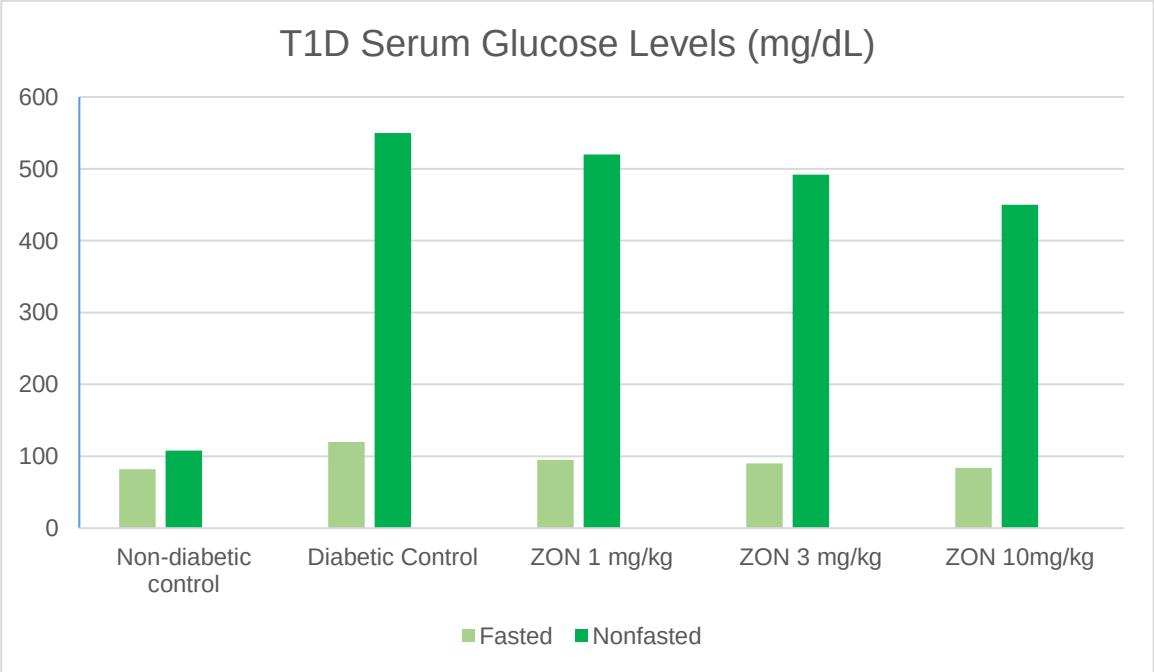
Concerning insulin secretion in RIN5f cells, they observed an increase of 3,5x in insulin secretion (10µg/mL of ZON in the medium) compared to a cell in a medium without the ZON (both in a medium with 25 mM/mL of glucose). They also verified that higher concentrations of ZON (30 µg/mL and 100 µg/mL) have proven to be cytotoxic.



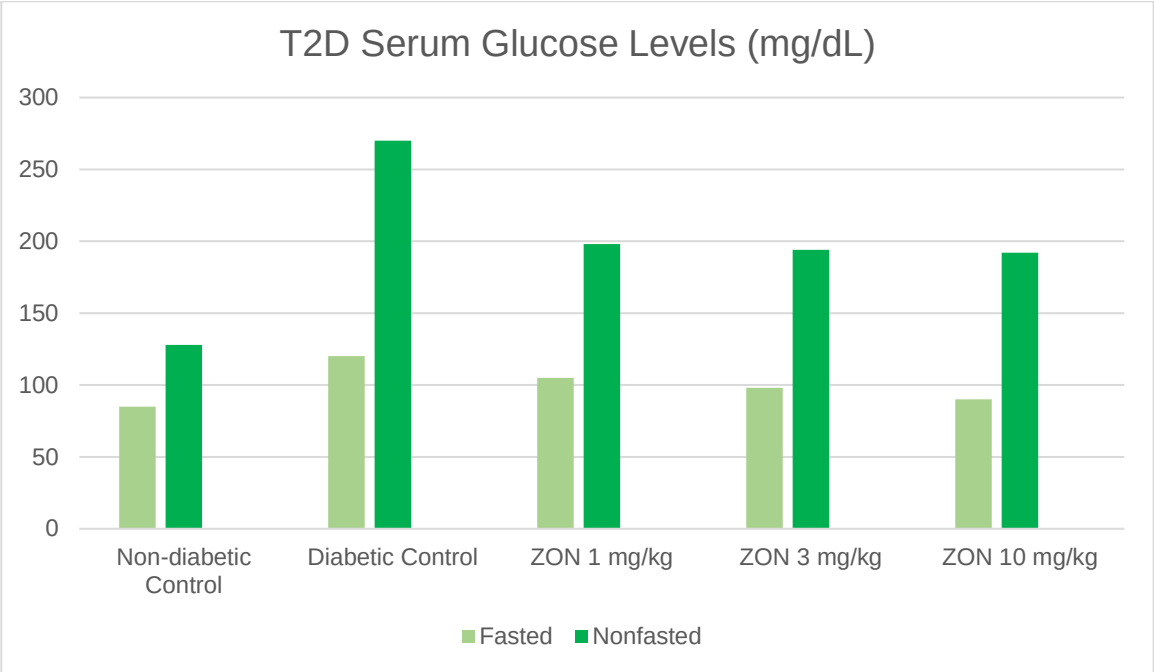
2 Umrani RD, Paknikar KM. (28)

At glucose concentration of 11 mM/mL in the medium, no major differences were observed.

Regarding the OGTT in rats, for the single-dose (3 and 10 mg/kg), serum glucose levels didn't suffered any statistically-significant alteration in T1D. In T2D, a significant lowering of glucose levels was observed. As for the repeated dose studies, they displayed promising results: although fasting serum insulin levels were not significantly altered (suggesting no risk of hypoglycemia as a result of ZON use), T1D showed increases in the order of 35% and an increase of 70% in T2D (10 mg/kg ZON in nonfasted rats). As for blood glucose levels, both T1D (20% and 26%, nonfasted and fasted, respectively) and T2D diabetic rats (29% and 21%, nonfasted and fasted, respectively) showed decreases with a ZON dose of 10mg/kg.



3Umrani RD, Paknikar KM. (28)



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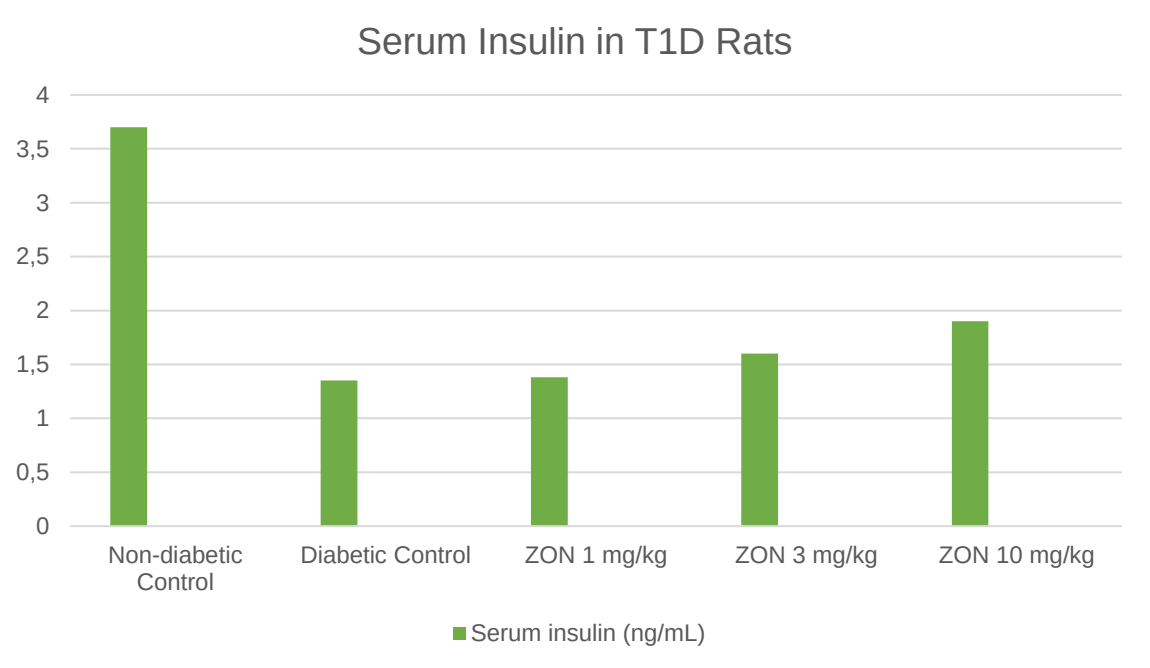
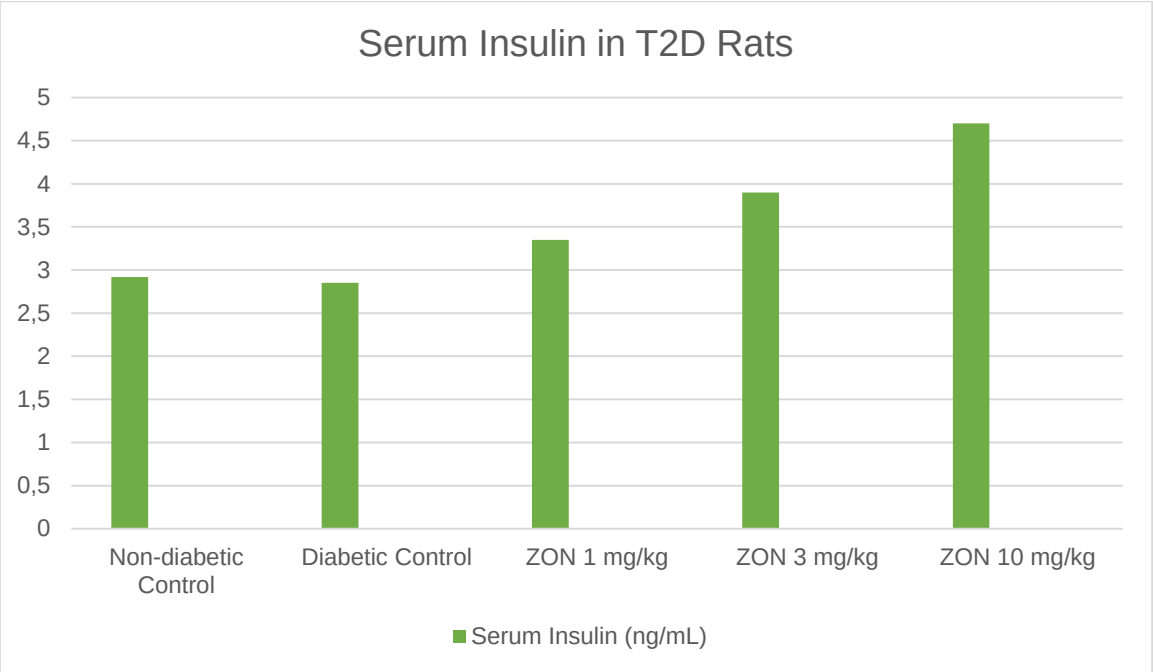


5 Umrani RD, Paknikar KM. (28)



6 Umrani RD, Paknikar KM. (28)

Results as promising were found also for serum TG and NEFA as well.

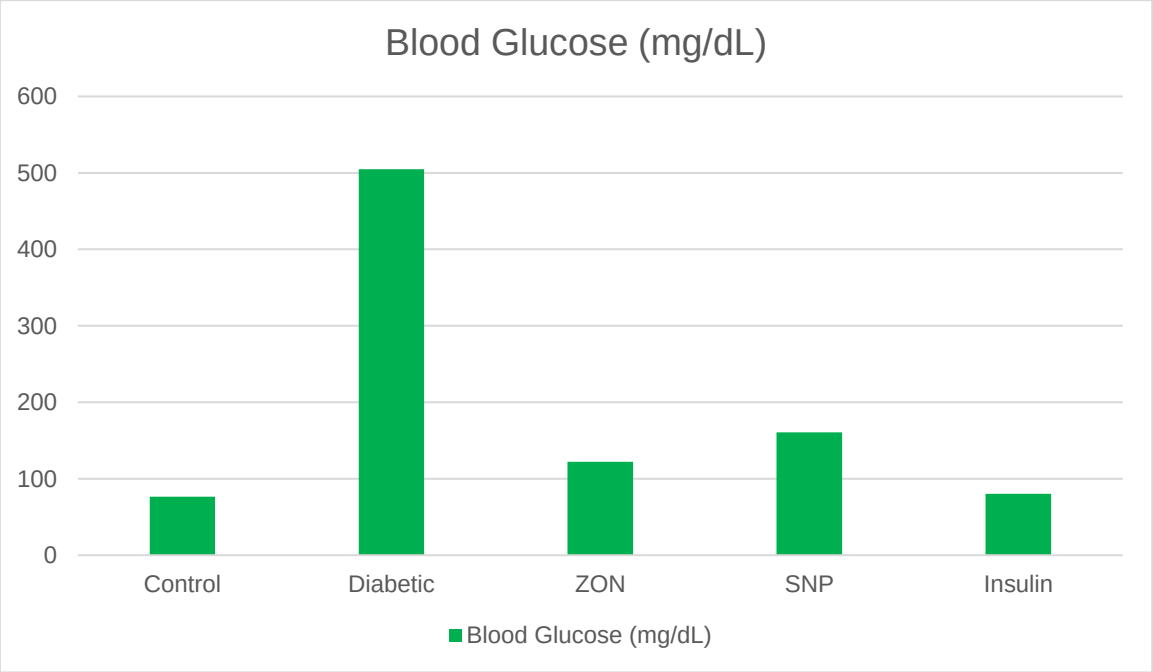
As for toxicity, they have found no major histological changes or sign of acute toxicity. ZON have showed to be safe even at concentrations 100 times superior of those that have showed to be effective, displayed not to be hemotoxic. It also appeared not to be cardiotoxic or hepatotoxic, since the values for glutamic pyruvate transaminase and glutamic oxaloacetic transaminase didn't seem to vary much.

We can infer directly by this study that both type 1 and type 2 Diabetic rats can benefit from ZON treatment, raising the urge for more and more solid studies regarding this matter. However, it is undeniable that the few number of specimens involved in the making of this study diminish its significance (a group consisted only of 6 individuals: 3 males and 3 females).

Other study, done by Alkaladi *et al.* in 2013[29] showed even more promising news regarding the use of zinc nanoparticles. Their study consisted on the usage of zinc oral nanoparticles (ZON) and silver nanoparticles (SNP) in diabetic albino rats, to

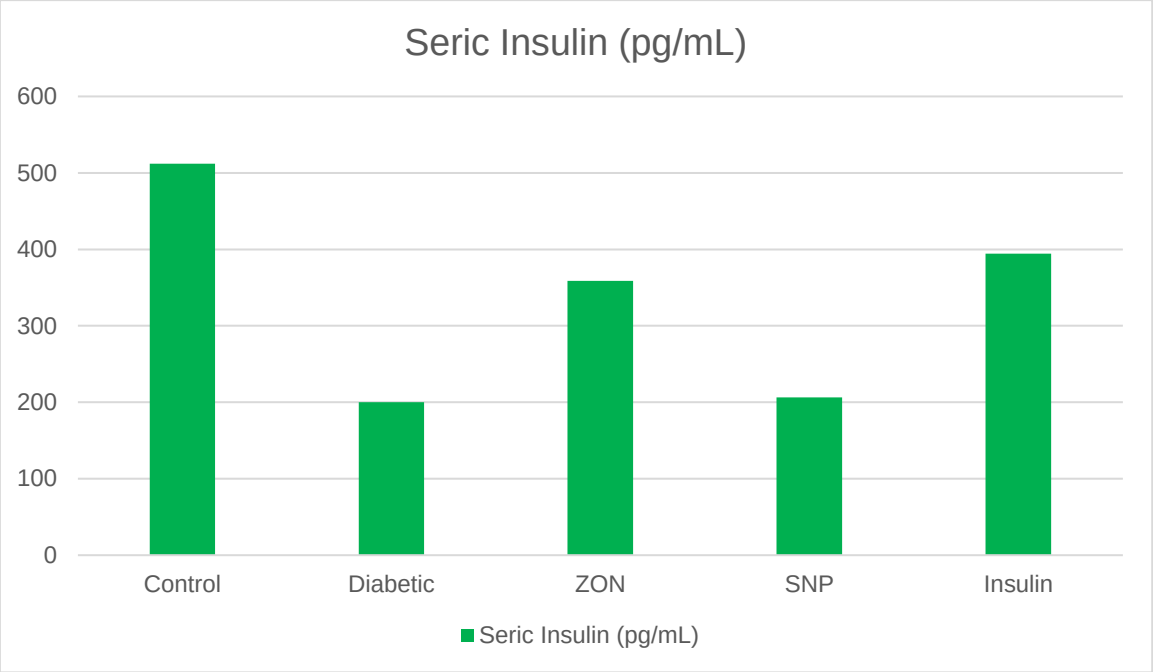
study their effects on blood glucose, glucose expression, insulin serum levels, glucokinase activity, glucokinase genes and glucose transporter GLUT-2.

As seen in the charts below, the results for their ZON were very satisfying and suggest a possible future use as an antidiabetic drug. The results were not so evident to the SNP. This test were done in comparison with control albino rats, streptozocin-induced diabetic rats and insulin-treated rats.



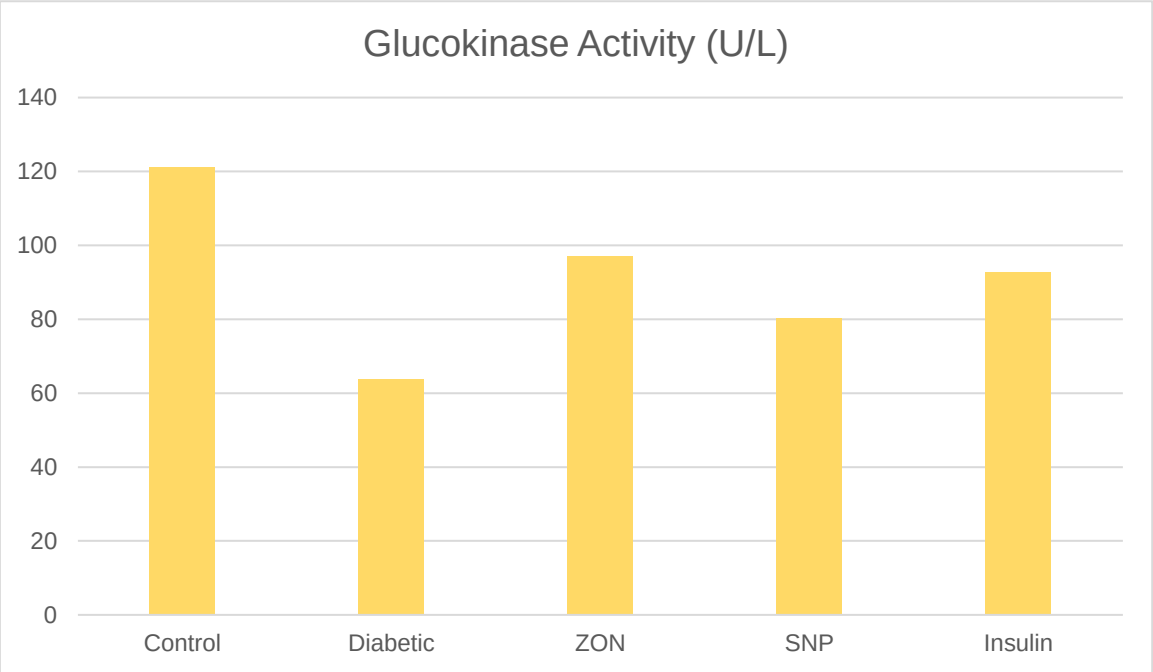
7 Alkaladi A, Abdelazim AM, Afifi M. (29)

As we can see in the chart, the nanoparticles were more or less effective keeping the blood glucose levels well below the value presented by non-treated diabetic rats, and not that far from the levels presented by insulin-treated rats.



8 Alkaladi A, Abdelazim AM, Afifi M. (29)

As for the blood insulin levels, it was clear that, although ZON performed well in increasing the values close to insulin-treated rats, SNP clearly failed, presenting values very similar to those observed in non-treated diabetic rats. Control rats exhibited values superior to those presented by both insulin-treated rats and ZON-treated rats.



9 Alkaladi A, Abdelazim AM, Afifi M. (29)

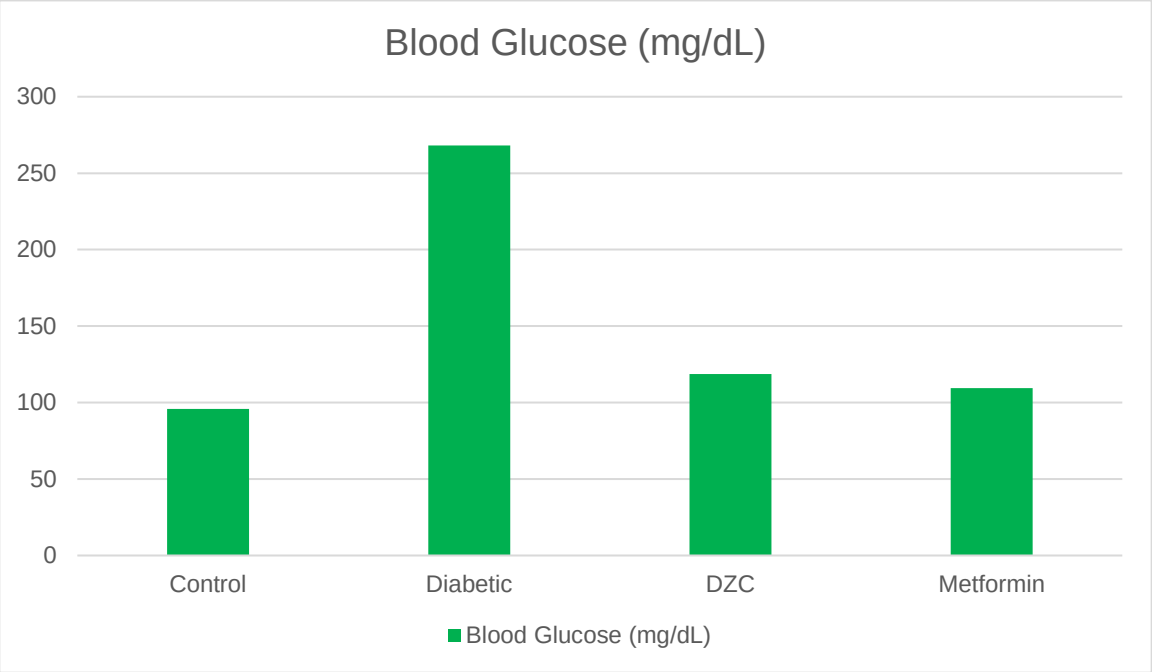
It was observed before that a decrease in both expression and activity of GK is often seen in diabetic models. [30] In terms of glucokinase activity, it can be observed a significant increase in ZON- and Insulin-treated rats. Even though SNP-treated rats showed an increased activity compared to non-treated rats, it wasn't as significant as the first two. We can still see that all of these values paled in comparison with those observed in control rats.

In 2015, Gopalakrishnan *et al.* [31] successfully produced a diosmin-zinc complex and tested it on Wistar albino rats to test it for antidiabetic activity.

Diosmin (dismetin-7-O-rutinoside) is a naturally occurring flavonoid in the pericarp of fruits like Meyer lemons and Buddha's finger fruit, [32] known to regulate glucose metabolism by increasing insulin secretion by β-cells in the pancreas. [33] This substance has been previously tested as a possible antidiabetic agent, and proved to be capable of effectively reducing blood glucose levels and minimize hyperglycemia secondary effects at a dose of 100 mg/kg. [34, 35]

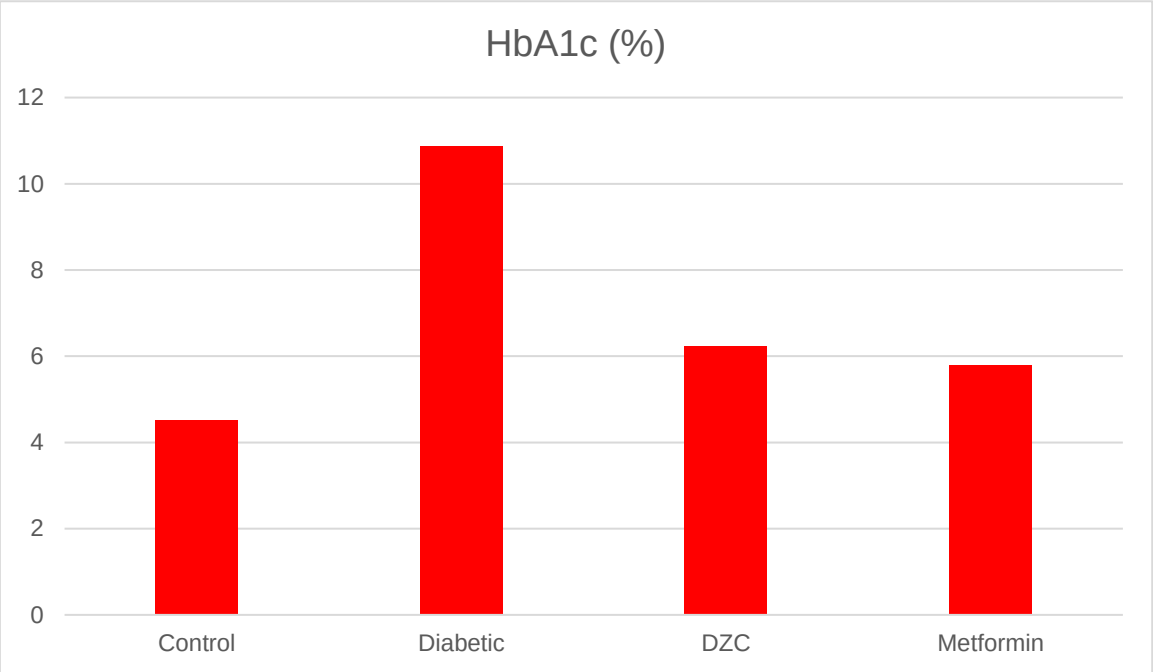
The processes of synthesis and structural elucidation and confirmation of the diosmin-zinc complex (DZC) is well explained. [31]

The following charts will show the results of the study regarding blood glucose, HbA1c, blood insulin and C-peptide values.



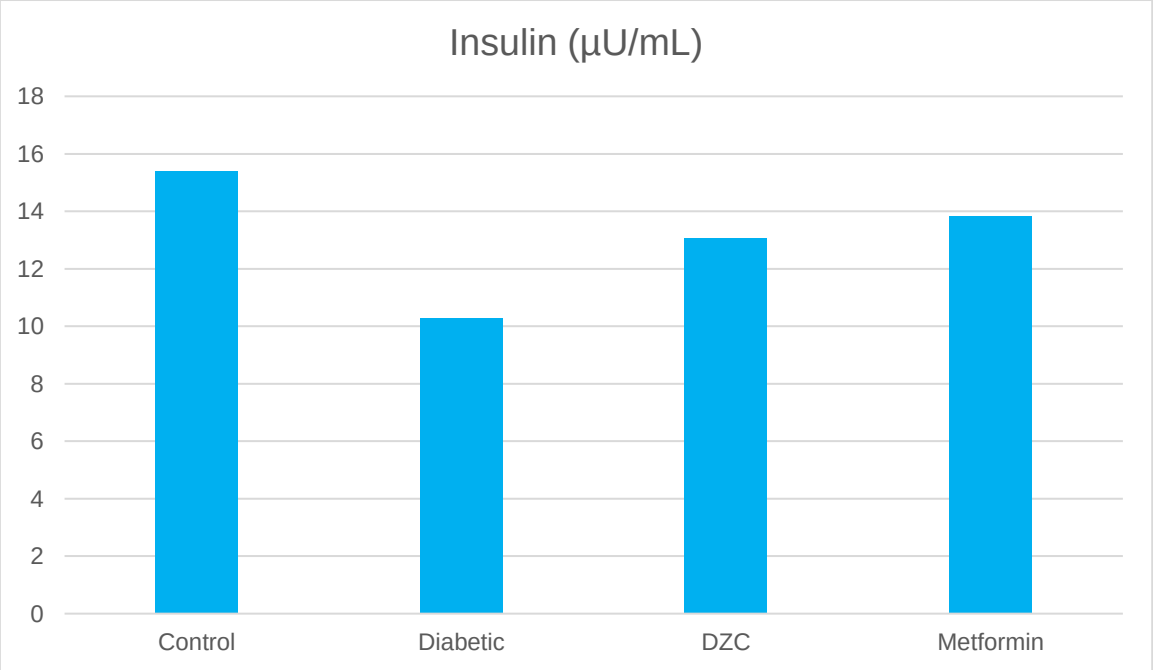
10 Gopalakrishnan V, Iyyam pillai S, Subramanian SP (31)

It is clear the DZC have showed results similar to those presented in metformin-treated rats, close to the control, and much lower than those in non-treated diabetic rats.



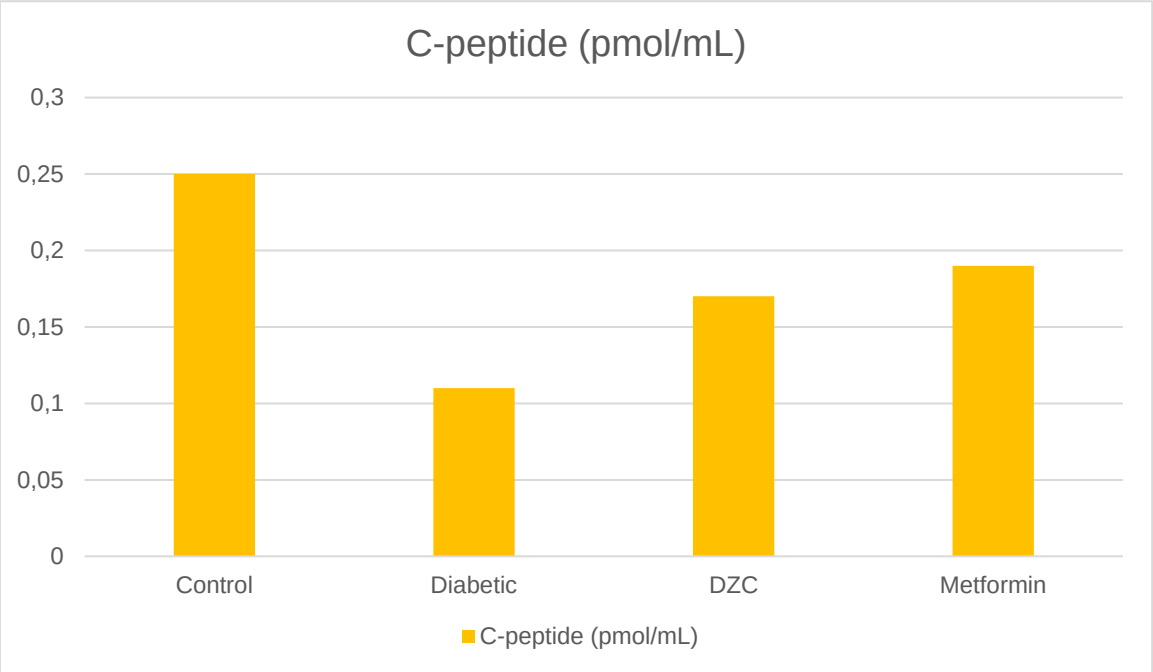
11 Gopalakrishnan V, Iyyam pillai S, Subramanian SP (31)

As for HbA1c, DZC continues to present similar results to metformin, even though they're slightly worse.



12 Gopalakrishnan V, Iyyam pillai S, Subramanian SP (31)

As seen before, DZC continues to show almost the same results as metformin.



13 Gopalakrishnan V, Iyyam pillai S, Subramanian SP (31)

C-peptide plasma values showed, consistently with the results from other markers studied, significant increase with both DZC and metformin.

Several other parameters were evaluated (such as liver glycogen, muscle glycogen, plasma protein content, blood urea, serum creatinine, AST, ALT, ALP, etc.) and they were all consistent with the result previously described: although metformin-treated rats showed the better results, the values in DZC-treated rats were always close, and a significantly better than the non-treated diabetic rats.

Even though the previous studies involving diosmin as an antidiabetic agent have proven to be successful, this DZC showed to be more potent (the results by using 100 mg/kg/day for 30 days oral diosmin have similar results to a dose of 20 mg/kg/day for 30 days of DZC), opening the road for further investigation and analysis of this complex, which might be another solution for diabetes management.

Discussion/Conclusion

With the expected rise of diabetes incidence in the world population, and the high morbidity it presents, presenting very large human and economic costs, it is a subject of great importance to find better, safer and cheaper ways to treat the disease.

The studies presented in this review show that is, at least, potential in the usage of zinc-based medicines to improve the treatment of diabetes. The particular case of studies involving the usage of nanotechnology have shown the most promising results.

There is, however, a lack of unquestionable proof of the link between zinc supplementation and improved serum insulin levels, glycaemia, etc.

Further studies are required, controlling the most important aspects of the patients' life, such as nutrition, physical activity and an adequate knowledge of their genome specificities. The size of the population in the studies reviewed also could be larger, and more ethnically diverse. Furthermore, it would be positive to have studies on the effects of zinc supplementation (either normal or with the usage of nanotechnology) on T1D, T2D, impaired glucose tolerance (also known as pre-diabetes) patients.

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