Research Article

Does the Simultaneous Use of Selenium and Vitamin E Improve Type 2 Diabetes Status?

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ABSTRACT

The aim of the present work was to investigate whether the antioxidant selenium (Se) and vitamin E (Vit E) treatment would impact on the onset of diabetes, compared to the currently available antidiabetic drug amaryl. We examined some biochemical parameters in serum including glucose, insulin, lactate dehydrogenase enzyme (LDH), albumin, total cholesterol, triglycerides and liver function enzymes (AST, ALT and ALP), also in liver tissue lipid peroxidation and glutathione reductase enzyme were examined. In addition, immunological studies were done including counting of splenocytes and thymocytes. Results showed that diabetes induces metabolic disorder and oxidative hepatopathy. This was indicated by the deviation in the above markers in blood, thymus, spleen and livers of diabetic rats. Oral administration of Se+Vit E after diabetes induction successfully modulated the diabetic increase in blood glucose, cholesterol, triglycerides, lipid peroxidation and liver function enzymes. Administration of these antioxidants ameliorates the decrease in insulin, albumin levels and glutathione reductase enzyme activity indicating their potential antidiabetic and antioxidant abilities. Immunologically, non-diabetic rats administrated Se+Vit E showed stimulation in splenocytes (1.9 folds). Rats supplemented with Se+Vit E before or after diabetic induction showed stimulation in splenocytes 2.2 and 1.9 folds, respectively. On the other hand treatment of diabetic rats with amaryl as a reference drug stimulated more splenocytes than that receiving Se + Vit E, either before or after STZ induction. In conclusion, treatment of diabetic rats with Se +Vit E could improve all the deviated diabetic biochemical parameters as compared with the available drug amaryl.

Keywords: Selenium – Vitamin E – Diabetes – Streptozotocin - Insulin – Glucose – Amaryl.

INTRODUCTION

Diabetes mellitus DM is a group of complex multisystem metabolic disorders characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues. The more prevalent form of diabetes is type 2. Based on current trends, more than 360 million individuals will have diabetes by the year 2030. Although the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 DM is rising much more rapidly because of increasing obesity and reduced physical activity [1].

Increased oxidative stress is an important contributor to the development and progression of diabetes and its complications. Diabetes usually occurs with increased production of free radicals or impaired antioxidant defense [2]. Under diabetic conditions, glucose is prone to oxidation resulting in the generation of hydrogen peroxide and reactive intermediates such as the hydroxyl radical [3]. Strategies to reduce the formation of oxidative stress are important in the treatment of DM [4].

Selenium is an essential trace element. Its importance is underlined by the fact that it is the only trace element to be specified in the genetic code - as selenocysteine[5]. Selenium is a key component of several functional selenoproteins (e.g., glutathione peroxidases), thioredoxin reductases, iodothyronine deiodinases and selenoprotein P that protect tissues and membranes from oxidative stress and control the cell redox status[6].

Epidemiological studies have shown an association between selenium and type 2 diabetes[7]. Because the incidence of type 2 diabetes is predicted to increase dramatically in the coming decades, it should be a priority to elucidate the effect of antioxidants such as Se and vitE on type 2 diabetes. Selenium has been shown to have insulin-like properties[8], which qualifies it as a potential antidiabetic agent.

Also, a number of investigations on the antidiabetic effects of supranutritional selenate doses have been carried out. Selenate (selenium oxidation state +VI) was shown to possess regulatory effects on glycolysis, gluconeogenesis and fatty acid metabolism, and metabolic pathways which are disturbed in diabetic disorders as reported by Mueller and Pallauf [9].

Immunologicaly speaking, the immune-enhancing effects of Se supplementation make it a promising complementary and alternative medicine modality for boosting immunity. Adequate levels of Se are important for initiating and regulating immune responses. On the other hand, Se deficiency has long been recognized to negatively impact immune cells during activation, differentiation, and proliferation, which is related to increased oxidative stress [10].

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Vit E, which has a critical role as a lipid-soluble antioxidant, is expressed on the cell membrane, and prevents lipid peroxidation in a variety of tissues in several pathological conditions, including diabetes, neurodegenerative disorders, cardiovascular diseases and cancer[11]. In the GK rat, a model for type 2 DM, Vit E supplementation significantly improved glycemic control, possibly by minimizing free radical damage to the pancreatic β-cells[12,13]. Another study using the obese Zucker rat, an animal that exhibits many of the features of type 2 DM showed improvements in glucose metabolism and insulin action by addition of Vit E that was mediated by a reduction in oxidative stress. They found that glucose-stimulated hyper-insulinemia and lipid peroxidation in the obese Zucker rat could be significantly reduced with dietary Vit E [14].

In a previous study we found that the coadministration of Se and Vit E to *Schistosoma* infected mice enhanced the humoral immune response [15]. The aim of the present study is to investigate the effect of orally simultaneous supplementation of Se as sodium slenate + Vit E on type 2 diabetic rats. Some biochemical parameters such as, insulin, glucose level, liver function parameters, glutathione reductase and lipid peroxidation will be evaluated. The count of splenocytes and thymocytes will be detected in treated and untreated diabetic rats and compared to the control group. The supplementations will be studied as alternative medical treatment and as antioxidants stimulators.

MATERIAL AND METHODS

I- Animals

Healthy female albino rats, weighing about 150-200 g were obtained from the animal house of the National Research Center, Cairo Egypt for the present study. Animals were maintained under standard environmental conditions, i.e. ambient temperature of (25 ± 2 °C), at 45-55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rats diet, water was supplied *ad libitum* [16]. All the studies were conducted in accordance with the Animal Ethical Committee of the National Research Center (09085).

II- Chemicals

All chemicals used in this study were of analytical grade. STZ was purchased from Sigma- Aldrich Company. The drug used as reference was amaryl (glimepiride as active ingredient). Total choleserol, triglycerides, albumin and lactate dehydrogenase activity were measured using kits purchased by Stanbio laboratory. AST, ALT and ALP activities were measured using kits delivered by Quimica Clinica Aplicada S.A., Spain.

Experimental design

Rats were divided into six groups, each group of 10 rats: G1: normal healthy rats.G2: diabetic rats. Experimental diabetes was induced by a single-dose (45 mg/kg, intraperitoneally, ip) streptozotocin (STZ) injection. G3:

rats orally treated with Se+ Vit E (2 μmole Se+250 mg Vit.E/kg/day) for three weeks. G4: rats orally treated with Se+Vit. E for three weeks then injected with STZ (prophylactic group). G5: rats injected with STZ then treated with Se+ Vit. E (treated group) for one month until glucose concentration was declined. G6: diabetic animals treated with amaryl drug (0.15mg/kg/day) for one month until glucose concentration was declined.

Induction of diabetes

Rats were fasted for 16 hrs then made diabetic by a single dose (45mg/kg bw) of streptozotocin injected intraperitoneally [17]. STZ was dissolved in 0.1 M citrate buffer (pH 4.5) [18]. The blood glucose level was checked before and 48 h after STZ injection to confirm the development of diabetes. The diabetic animals were stabilized for three days and the next day (day 0) experiment was started. Blood samples were taken from lateral tail vein and glucose was measured by the blood glucose monitor (Bionime G M 100, produced in Taiwan). Only those animals which showed blood glucose levels >250 mg/dL were separated and used for the study. The last dose of any treatment was given 24 hours before sacrificing the rats. The animals were fasted 14 hours before sacrificing in order to minimize the variations in lipid patterns and to measure the fasting glucose. At the end of the experimental period, blood was collected from the tongue after subjecting them to ether anesthesia and used for serum separation [19]. Sera were obtained by centrifugation at 3000 rpm for 15 min at 4°C and used for the biochemical analysis.

Liver homogenates

1 gm of the liver of each animal was homogenized in 10 mL bidistelled water, using a glass-teflon homogenizer for the determination of total protein and glutathione reductase activity, and 0.2 gm of liver was homogenized in 2 mL 10% trichloroacetic acid (TCA) for the determination of lipid peroxidation.

Biochemical assays

Insulin was determined in sera[20], total cholesterol and triglycerides were estimated as a quantitative enzymatic, colorimetric method in serum[21, 22] respectively. Serum albumin was measured quantitatively by a colorimetric method[23]. Lactate dehydrogenase activity was kinetically measured through the oxidation of lactate to pyruvate in the presence of NAD[24]. Alkaline phosphatase (ALP) was measured colorimetricaly[25]. Serum activities of AST and ALT were measured colorimetrically[26]. Total protein in liver homogenates was measured according to Beirut method[27]. Glutathione reductase activity was measured in liver homogenates by kinetic assay[28]. Lipid peroxidation was determined by measuring the formed MDA (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method[29].

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Immunological studies

Detection of the total number of splenocytes and thymocytes were done using trypan blue staining. The spleen and thymus were prepared as mentioned by Maghraby [30]. Equal volume (0.1 ml) of whole suspension and trypan blue were mixed and examined under LEITZ microscope using Neubaur haemocytometer. Viable lymphocytes exclude the dye while dead cells appear blue. Viable lymphocytes were counted according to the equation:

$$\text{Viable lymphocytes} = N \times Y\, 2 \times 10^{4} = 10^{6}/\,ml.$$

Where N: number of viable cells per 16 large squares. Y: the volume of cells suspension.

Statistical analysis

Data were analyzed with SPSS (Statistical Package for the Social Sciences) version 9.5 software package (USA). Results were expressed as mean ± S.D. A p value of 0.05 or less was considered statistically significant.

RESULTS

Data in Table (1) showed a significant increase in glucose concentration of diabetic rats (G2) as compared to control group (G1). This increase in blood glucose level in diabetic rats was ameliorated by treating the animals with either amaryl or Se+ Vit E. On the other hand prophylactic supplementation of Se+ Vit E showed less effect on elevated increase in glucose level.

Results in Table (1) revealed a high significant decrease in insulin level in diabetic rats. This decrease was partially corrected when using the drug amaryl. The prophylactic use of Se+ Vit E was less effective than the drug, however no great change was measured in the treated group when compared with the effect of the drug.

Amaryl was much less effective on the increase in serum LDH activity than either both treatments of Se+ Vit E (Table 1). All treatments reduced the negative effect of diabetes on albumin contents (Table 1).

Data in Table (2) showed that, treatment with Se + Vit E induced better effect on serum total cholesterol concentration and triglycerides content in diabetic rats when compared with either the prophylactic or the treatment with the drug amaryl.

The increase in lipid peroxidation level due to diabetes was improved in liver tissue homogenates of treated group with Se+ VitE, than both of the prophylactic and the amaryl groups (Table 3). Best recovery in glutathione reductase activity was recorded in the animal group treated with Se+Vit E. Although the enzyme activity in the prophylactic group was improved when compared to the diabetic animals, this amelioration is far from the normal level.

Data in Table (4) also showed that the amelioration in serum AST, ALT and ALP activities in the group treated with amaryl was less than the two other treatments.

As shown in fig (1) selenium and vitamin E stimulated the thymocytes number even in non-diabetic rats where an increase in the number of thymocytes was recorded in the animal goups supplemented with Se+ Vit E. The highest increase in the number of thymocytes was in the diabetic group simultaneously treated with the antioxidants Se and Vit E after STZ induction. On the other hand, treatment of diabetic rats with amaryl stimulated the number of splenocytes more than that in rats receiving Se + Vit E, either prophylactic or after STZ induction (Fig 2).

Table 1: Effect of Se+Vit E on some selected serum parameters of different studied groups

Groups → Parameters ↓	Normal Control (G1)	Diabetic (G2)	Normal Se+ Vit.E (G3)	Prophylactic Se+Vit.E (G4)	Treated Se+Vit. E (G5)	Amaryl drug (G6)
Glucose(mg/dL)	84.13±4.63	461.96±58.45^{a}	84.14±4.75^{n*}	250.66±26.28^{a*}	103.03±9.20^{a*}	109.71±8.94^{n} *
Insulin (ng/ml)	5.52±0.33	1.49±0.23^{a}	5.52±0.21^{n} *	2.30± 0.14^{a*}	3.13±0.13^{a} *	2.95±0.41^{a} *
LDH (U/L)	341.66 ±23.51	776.72 ±40.39^{a}	335.93±26.24^{n*}	484.43±22.27^{a} *	423.18±33.19^{a} *	578.38±22.15^{a*}
Albumin (g/dL)	5.17 ±0.68	3.25 ± 0.42^{a}	4. 91 ±0.40^{n*}	3.87±0.59^{a}#	4.21 ±0.28^{b***}	4.63 ±0.22^{n**}

Data are presented as mean ± SD of 6 rats in each group. ^{a}P ≤ 0.0001, ^{b} P ≤ 0.01 , n , not significant compared with control group. * P ≤ 0.0001, **P ≤ 0.001, ***P ≤ 0.01, #, not significant compared with diabetic group.

Table 2: Effect of Se+Vit E on some lipid profile parameters in sera of different experimental normal and diabetic groups.

Groups → Parameters ↓	Normal Control (G1)	Diabetic (G2)	Normal Se+ Vit.E (G3)	Prophylactic Se+Vit.E (G4)	Treated Se+Vit. E (G5)	Amaryl drug (G6)
Total cholesterol (mg/dL)	75.66+4.61	109.04±9.44^{a}	75.71 ±3.91^{n*}	85.17 ±4.60^{b*}	76.23±5.18^{n*}	92.36±6.32^{a} * *
Triglycerides (mg/dL)	81.92+7.04	156.72±7.94^{a}	81.70±6.31^{n} *	122.07±6.83a *	97.43±3.59^{c} *	112.55±11.27^{a} *

Data are presented as mean ± SD of 6 rats. ^{a} P ≤ 0.0001, ^{b} P ≤ 0.001, ^{c} P≤ 0.01, n , not significant compared with control group. * P ≤ 0.0001, **P≤ 0.001 compared with diabetic group.

Table 3: Effect of Se+Vit E on lipid peroxidation and glutathione reductase enzyme in liver tissue of different experimental normal and diabetic groups.

Groups → Parameters ↓	Normal Control (G1)	Diabetic (G2)	Normal Se+ Vit.E (G3)	Prophylactic Se+Vit.E (G4)	Treated Se+Vit. E (G5)	Amaryl drug (G6)
MDA (ng/g tissue)	9.20±1.72	19.70±2.13[a]	10.32± 2.15[n] *	14.09 ±2.01[b] **	11.36 ±1.89[n] *	14.21 ±1.92[b] **
GR (nmol/min./mg protein)	11.48±1.73	3.06±0.91[a]	11.25±1.4 4[n] *	6.04±2.16[a] ***	9.96±1.09[n] *	8.29±1.74[c]*

Data are presented as mean ± SD of 6 rats. [a] P ≤ 0.0001, [b] P ≤ 0.001, [c] P≤0.01, n , not significant compared with control group. * P ≤ 0.0001, **P≤ 0.001, ***P ≤ 0.01 compared with diabetic group.

Table 4: Effect of Se+Vit E on liver function enzymes in sera of different experimental normal and diabetic groups

Groups → Parameters ↓	Normal Control (G1)	Diabetic (G2)	Normal Se+ Vit.E (G3)	Prophylactic Se+Vit.E (G4)	Treated Se+Vit. E (G5)	Amaryl drug (G6)
AST (U/ml)	34.34±2.77	95.82 ±3.93[a]	34.06±3.16[n] *	61.99 ±2.65[a]*	51.42 ±2.46[a] *	76.51 ±3.78[a]*
ALT(U/ml)	40.11 ±2.11	96.92±2.27[a]	39.19±2.70[n]*	68.03±4.72[a]*	53.09±6.17[a] *	67.54 ±5.18[a]*
ALP(IU/L)	35.62±3.08	97.86±12.53[a]	35.91±3.96[n]*	57.2 4±8.44[a]*	46..03 ±3.65[b]*	78.83 ±6.76[a]**

Data are presented as mean ± SD of 6 rats. [a] P ≤ 0.0001, [b] P ≤ 0.01, n , not significant compared with control group. * P ≤ 0.0001, **P ≤ 0.001 compared with diabetic group.
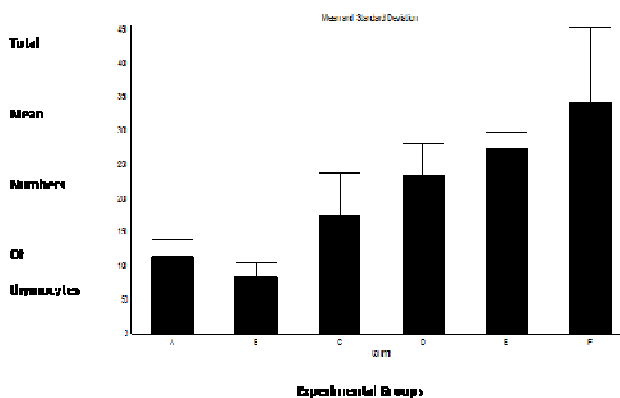
Figure 1: The mean number of thymocytes in A:-ve control; B: +ve control; C: amaryl; D: normal Se+Vit E (no diabetis); E: prophylactic Se+Vit E+diabetic; F-Se+Vit E treated diabetic
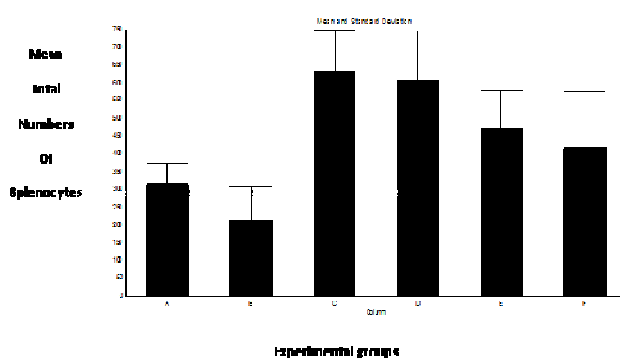
Figure 2: The mean number of splenocytes in A:-ve control; B: +ve control; C: amaryl; D: normal Se+Vit E no diabetis; E: prophylactic Se+Vit E+diabetic; F-Se+Vit E treated diabetic

DISCUSSION

Being essential or not, trace elements have been identified for long time as potential candidates for treatment or to mitigate severity of complications of some metabolic disorders including diabetes. Does selenium promote type 2 diabetes, or improve glucose metabolism or act as insulin mimic compound? How can selenium play a role in diabetes? All these questions arose in the last decades, but precise mechanistic evidence is lacking to explain the clinical and epidemiological data that whether selenium plays a negative or a positive role in type 2 diabetes.

There are animal [31-33] and case control studies[7,34,35] that suggest selenium may improve glucose metabolism. Vitamin E is known to have a synergistic effect as antioxidants in animals and human. In the present work we studied the combined effect of selenium together with Vit E both as antioxidant dietary intake.

Oral supplementation of selenium together with Vit E either as prophylactic or after STZ induction showed an improvement in insulin level in diabetic rats when compared to diabetic non treated group. Also the fasting blood sugar was decreased in diabetic rats due to these treatments when compared to diabetic non treated animals. Several explanations can be discussed in view of these results together with previous studies by other researchers.

Selenium has been shown to have insulin-like properties[8], which qualifies it as a potential antidiabetic agent. It is one of the hypotheses which attribute the anti-diabetic mechanism of selenium species to their insulin–like properties. This was discussed in a previous work in view of the results of the effect of sodium selenate in streptozotocin-induced diabetic rats [36].

Selenate administration in supranutritive doses to rats with streptozotocin – induced type 2 diabetes led to a sustained correction of their diabetic status including the decrease of the elevated blood glucose concentration and considerable changes in the expression of normally expressed glycolytic and glyconeogenic marker enzymes[37].

From *in vivo* experiments and *in vitro* studies with tissue cultures, it was concluded that enhanced phosphorylation reactions at the β subunit of the insulin receptor and further components of the insulin signalling cascade are responsible for the so-called insulinomimetic properties of selenate [38,39].

Selenate (selenium oxidation state +VI) was shown to possess regulatory effects on glycolysis, gluconeogenesis and fatty acid metabolism, metabolic pathways which are disturbed in diabetic disorders. An enhanced phosphorylation of single components of the insulin signalling pathway could be shown to be one molecular mechanism responsible for the insulinomimetic properties of selenate. In type II diabetic animals, a reduction of insulin resistance could be shown as an outcome of selenate treatment [9].

Another hypothesis is the antioxidant effect of both Se and Vit E. The present results which are supported by several published works[12,13] are an evidence for the antioxidant responsibility of Se and Vit E in modulating the glucose and insulin levels in diabetic rats by scavenging the produced free radicals. Glutathione reductase (GR) activity and the level of lipid peroxidation (LPO) as markers for antioxidant status were measured in the liver homogenates since the liver is the main organ involved in detoxifying free radicals and thus oxidative stress in liver happens in the early stages of diabetes[4].

The data of the antioxidant status available in the present study, which are in harmony with the results of insulin and blood glucose levels, may enforce the hypothesis of the antioxidant effect of Se and Vit E on glucose level. The antioxidant effect of Vit E was discussed by Paolisso[40], who investigated the potential metabolic benefits deriving from daily vitamin E administration in type II diabetic patients. They found that long term vitamin E administration reduced plasma glucose, triglycerides, FFAs, total cholesterol, low-density lipoprotein cholesterol, and apoprotein B levels but did not affect beta-cell response to glucose. Glycated proteins (HbA1c) levels were also significantly lowered.

In the present study LPO levels were decreased in groups treated with both Se +Vit E. Also the activity of liver homogenates GR enzyme was improved due to these supplementations. These data are in accordance with several published results who measured a decrease in LPO level in diabetic rats due to antioxidant supplementations [41,42]. The correction of the increased level of LPO by Se and Vit E as antioxidants explains that at least part of the causes why the liver is affected in hyperglycemic rats is attributed to the oxidative stress. In fact, as discussed by Di Naso et al. [4], a clear link between oxidative stress and diabetes exists. Liver plays an important role in glucose metabolism, and in a chronic hyperglycemic state, liver oxidative stress is considered a relevant process. Oxidative stress induced by hyperglycemia leads to liver cell damage because liver is subjected to reactive oxygen species ROS-mediated injury

in diabetes. A significant increase of oxidative stress biomarkers, ROS and a reduction in antioxidant enzymes in plasma and liver of diabetic rats were corrected when using antioxidant [43]. In the present study the increase in liver enzymes activity (AST, ALT and ALP) as well as the lipid profile represented by triglycerides and cholesterol is an evident for the effect of diabetes on the liver. The correction of these increase by Se and Vit E, as antioxidant explains, that the harmful effect of hyperglycemia to the liver is, at least part of it, due to oxidative stress induced by diabetes.

A third hypothesis which can be discussed in view of the present study is the effect of selenium and Vit E on the immune system in diabetic rats. The biological effects of Se are mainly exerted through its incorporation into selenoproteins as the amino acid, selenocysteine (Sec). Several of these and other less well-characterized selenoproteins have been shown to be expressed in nearly all tissues and cell types, including those involved in innate and adaptive immune responses[44-46]. Selenoproteins are thought to play roles in the effects of altered Se status on immune responses [46,47]. Se deficiency has been implicated in accelerated disease progression and poorer survival among populations infected with human immunodeficiency virus[48]. However, effects of more subtle changes in Se status are less well defined and definition of the functions and underlying mechanisms is lacking. Se has been shown to cause immune response promotion[49] and cytokine production[50].

In a previous study it was found that Se and Vitamin E enhanced the humoral immune response in *Schistosoma mansoni* infected mice [15]. In the present study an administration of Se and Vit E before or after inducing diabetes significantly enhanced thymocytes as well as splenocytes production, which was in agreement with many workers, who studied the direct immune effect of selenium and vitamin E. These studies showed that both Se and vitamin E deficiencies may affect both the maturation of thymocytes and splenocytes subpopulations and the functional and proliferative capabilities of the peripheral lymphocytes [51]. The present results are also in agreement with Malinin et al [52]. Here again we can attribute the enhancement of immune system by Se and Vit E to their antioxidant behavior. GSH levels were measured as an indicator of redox status in the primary porcine splenocytes after Se supplementation[53]. GSH, a ubiquitous thiol-containing tripeptide, is the major intracellular antioxidant with multiple biological functions, including the maintenance of thiol group of the cysteine residue [54] and the reduced form of many other molecules[55]. Intracellular GSH concentration in unstimulated, or mitogen-stimulated splenocytes was found to increase after selenium supplementation.

CONCLUSION

According to the present data and the published works which are in agreement with us and on the other hand

the publications, which are not parallel to them [56] , it can be concluded that, Selenium can play a dual role in diabetes according to the dose used and its chemical form. If Se will be supplemented to human the level of the body Se must be known specially before long term use.

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