International Scholars Journals

International Journal of Urology and Nephrology ISSN 2756-3855 Vol. 9 (1), pp. 001-006, January, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

The effect of vitamin C and cobalt supplementation on antioxidant status in healthy and diabetic rats

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Accepted 18 August, 2020

In the present study, liver, lung, heart and kidney superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activities, lipid peroxidation, nitrite and vitamin C levels were investigated in diabetic rats. Diabetes induced in rats by streptozotocin (STZ) and the treated rats were received 1 g/l vitamin C with 0.5 mM $CoCl_2$ in drinking water for eigth weeks. In all tissues, superoxide dismutase, glutathione peroxidase, catalase activities, lipid peroxidation and nitrite levels were significantly increased in diabetic rats at the end of 8th week ($p < 0.05$) whereas vitamin C level was decreased compared to those of controls. Cobalt with vitamin C treatment of diabetic rats resulted in partial restoration of SOD and CAT activities, thiobarbituric acid reactant substances (TBARS), vitamin C and nitrite levels at all times studied, whereas treatment did not significantly change GSH- Px activity compared to diabetics. These results suggest that cobalt with vitamin C, effectively but could not completely restore the altered endogenous defense systems in diabetic rat liver, lung, heart and kidney tissues.

Key words: Cobalt, antioxidant enzymes, diabetes, lipid peroxidation, tissue.

INTRODUCTION

Hyperglycemia causes increased production of free radicals via autoxidation of glucose and non-enzymic pro-tein glycation that may lead to disruption of cellular functions and oxidative damage to membranes (Wollf et al., 1991; Miyata et al., 1998; Baynes and Thorne, 1999; Osawa and Kato, 2005). Evidence is accumulating which suggests that toxic reactive oxygen species play a crucial role in diabetes (Darley-Usmar and Halliwell, 1996; Giardino et al., 1998). The levels of reactive oxygen species are controlled by antioxidant enzymes including glutathione peroxidase (GSH-Px) (EC 1.11.1.9), catalase (CAT) (EC 1.11.1.6) and superoxide dismutase (SOD) (EC 1.15.1.1). There is increasing evidence that, in cer-tain pathological states, the increased production and/ or ineffective scavenging of reactive oxygen species may play a crucial role in determining tissue injury (Kehrer, 1993; Ceriello, 2000).

Direct evidence that uncontrolled diabetes may be associated with alterations in the generation and/or scavenging of reactive oxygen species. The nutritional antioxidants include vitamin C (ascorbic acid) is a hydro-philic vitamin. As a scavenger of reactive oxygen species, ascorbate has been shown to be effective against superoxide radical anion, hydrogen peroxide, the hydro-xyl radical and singlet oxygen. In aqueous solutions, vitamin C also scavenges reactive nitrogen oxide species efficiently, preventing the nitrosation of target molecules. On the other hand, in recent years, numerous reports have described the *in vitro* and *in vivo* insulin-like activity of several elements (Tian and Lawrence, 1998; Sakurai and Adachi, 2005; Hiromura and Sakurai, 2008). In streptozotocin (STZ) induced diabetic rats, 2 mM cobalt chloride ($CoCl_2$) added to the drinking water resulted in the decrease of plasma glucose levels and also an increase in glucose transporter (GLUT-1) mRNA content of ventricular myocardium, renal cortex, skeletal muscle, cerebrum and liver (Ybarra et al., 1997). Treatment with $CoCl_2$ may also reduce hepatic glucose output and thus lower blood glucose. Insulin treatment of diabetic rats resulted in complete reversal of all the foregoing altera-tions in tissue antioxidant status (Wohaieb and Godin, 1987). Cobalt is considered an essential nutritional trace element and has therapeutic value in pharmacological doses and its oral administration improves the altered glucose homeostasis of the diabetic state (Ybarra et al., 1997; Saker et al., 1998).

Also, in our previous study we showed that blood glucose levels of cobalt chloride with a combination of ascorbic acid treated diabetic rats, exibited significant reduction at the end of 6th week (46%) approaching nearly normal levels (Yıldırım and Büyükbingöl, 2002). Regarding to previous results the aim of this study was to investigate the effects of vitamin C with a combination of cobalt chloride on streptozotocin induced diabetic rat liver, lung, heart and kidney tissues. Therefore, the changes in lipid peroxide, vitamin C and nitrite levels and the activities of SOD, GSH-Px, CAT were studied, which are associated with the detoxication functions in these tissues.

MATERIALS AND METHODS

Materials

Streptozotocin, nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), bovine serum albumin, glutathione reductase, 2- thiobarbituric acid (TBA), quercetin, 2,4-dinitrophenilhydrazin were purchased from Sigma Chemical Co. Ascorbic acid, cobalt (II) chloride, malondialdehyde, trichloroacetic acid (TCA) were from Merck Company. All other materials used were of analytical grade.

Animal treatment

Male Sprague Dawley rats initially weighing 180-200 g were individually housed in suspended stainless steel cages in a room maintained at 21 ± 1°C with a 12 h light/dark cycle. Diabetes was induced by a single intravenous injection of STZ, at a dose of 40 mg/kg (prepared in 0.1 M citrate buffer, pH 4.5). Control (healty) animals were injected with the equivalent amount of buffer intravenously. Diabetic state was confirmed by measuring glucose in blood obtained from the tail vein using Reflolux (Boehringer Mannheim, East Sussex, UK) glucometer and test strips. The minimal blood glucose value accepted for a diabetic rat was 14 mM. Animals were randomly assigned to 4 groups for each week:

(1) Healty group (C).
(2) Diabetic group (D).
(3) Vitamin C and cobalt treated healty group (VitC+Co).
(4) Vitamin C and cobalt treated diabetic group (D+ VitC+ Co).

For the vitamin C and cobalt treated animals, 1 g/l ascorbic acid and 0.5 mM cobalt (II) chloride were given daily in the drinking water for 8 weeks. At indicated times, the rats were anaesthetized with urethane (750 mg/kg body weight, intraperitoneally). Liver, lung, heart muscle and kidney tissues of each rat was immediatelly removed, cleaned of gross adventitial tissue, blotted dry, wrapped in aliminum foil and then stored at -80°C before assay within 3 weeks.

Tissue sample analysis

Tissue samples, which were sliced and from which veins and membranes had been removed, were homogenized in 3 volumes of % 1.15 KCl at 4°C for 30 s (2x 15 sec with 15 s cooling interval) at 2,000 r.p.m. using a teflon glass pestle with Heidolph homogenizer (Type 50110, Germany). Cell debris was removed by centrifugation at 4°C and 10,000 g for 20 min. The resultant supernatant was used for measurement of TBARS level and antioxidant enzymes activity.

A simple assay system for SOD was based on the inhibitory effects of SOD on the spontaneous oxidation of quercetin (Kostyuk and Potapovich, 1989). The oxidation rate of quercetin was determined by observing the absorbance changes at 406 nm. One U is the amount of SOD required to inhibit the initial rate of quercetin oxidation by 50%. The assay mixture contained 0.9 ml of 16 mM phosphate buffer (pH 9.2) including 0.890 mM TMEDA (N,N,N',N'- tetramethylethylenediamine) and 0.0890 mM EDTA, 0.5 ml of 10,000 g supernatant (or water) and 0.5 ml of 0.3 mM quercetin in a final volume of 1.0 ml. Quercetin was added to start the reaction and after rapid mixing, the decrease in absorbance at 406 nm was followed for 20 min.

Selenium-dependent glutathione peroxidase (GSH-Px) estimations were based on the following principle: GSH-Px catalyses the oxidation of glutathione by hydrogen peroxide and the rate of change of absorbance during the conversion of NADPH to $NADP^+$ was recorded spectrophotometerically at 340 nm for 3 min as described by Lawrence and Burk (1976). GSH-Px activity was expressed as micromoles of NADPH oxidized to $NADP^+$ per minute per milligram protein using a molar extinction coefficient for NADPH at 340 nm of 6.22×10^6. Selenium dependent GSH-Px was assayed in a 1 ml cuvette containing 0.760 ml of 0.1M phosphate buffer (pH 7.0), 0.050 ml cytosolic fractions, 0.050 ml of 0.1mM NADPH, 0.050 ml of 1.5 U/ml glutathione reductase and 0.040 ml of 4.0 mM glutathione in a total volume of 0.950 ml. The reaction was started by the addition of 0.050 ml of 3.0 mM H_2O_2 and the conversion of NADPH to $NADP^+$ was monitored by continuous recording of the absorbance change of the system at 340 nm.

CAT activities of heart and aorta were measured by the method of Aebi (1987) at 240 nm and expressed as the first order rate constant (*k*) per milligram protein. CAT was assayed by incubating 10,000 g supernatant fractions with Triton X-100 (20:1, v/v) for 20 min at 4°C. From this mixture 100 µl was diluted to 10 ml with 50 mM phosphate buffer, pH 7.0. In a 1 ml cuvette, 500 µl of diluted solution was placed and the reaction was started by adding 250 µl of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was determined from the absorbance changes at 240 nm.

Substances that react with thiobarbituric acid were measured as described by Uchiyama and Mihara (1978) using the same extraction medium as that for antioxidant enzyme assays. TBARS react with products of lipid peroxidation, mainly malondialdehyde, producing a coloured compound which can be measured at 535 nm. Therefore this method is indirect evidence of the process of lipid peroxidation. Samples of tissue supernatants (0.5 ml) were added to test tubes containing 3.0 ml of phosphoric acid (1%) and 1.0 ml of thiobarbituric acid (0.6% w/v) and the reaction mixture was heated at 95°C for 45 min. After cooling the tubes in ice, 4.0 ml of n-butanol was added to each tube, vortexed for 20 s and centri-fuged at 5,000 g for 5 min. The n- butanol layer was used for spectrophotometric measurement at 535 nm using a molar extinction coefficient of 1.56×10^4 and the results were expressed as nmol TBARS per mg protein.

The tissue sample homogenized as mentioned above (0.5 ml) was incubated with nitrate reductase (EC 1.6.6.2) from *Aspergillus* sp. (50 mU/100 µl of sample) with NADPH (final concentration, 80 µmol/l) diluted in 20 mmol/l Tris buffer (pH 7.6) for 30 min at room temperature for nitrate reduction (Guevara et al., 1998). After the reduction, 5% (w/v) $ZnSO_4$ was added for deproteinization. Then this mixture was cetrifuged at 5000 x g for 10 min. The samples nitrite levels were measured by Griess reaction (Green et al., 1982) The absorbance at 540 nm was measured. Nitric oxide levels were calculated from the standard curve constructed using known nitrite concentrations. Tissue nitrite levels were expressed as µmol g^{-1} wet

Table 1. Comparison of parameters of rat liver with control, diabetic, vitamin C with cobalt treated control and diabetic animals.

Organ	Parameter	Control	Vıtamın C + Cobalt	Dıabetıc	Dıabetıc + Vıtamın C + Cobalt
LIVER	SOD activity (U mg^{-1} protein)	161±7	170±7	248 ±10∗	189 ± 8•
	GSH-Px activity (mol NADPH min^{-1} mg^{-1} protein)	0.29 ± 0.029	0.30 ± 0.039	0.59 ± 0.09∗	0.49 ± 0.054∗
	CAT activity (k $s^{-1}mg^{-1}$ protein)	0.23 ± 0.017	0.29 ± 0.018	0.61 ± 0.019∗	0.35 ± 0.044∗•
	MDA (nmol TBARS mg^{-1} protein)	0.060 ± 0.0054	0.065 ± 0.0036	0.136 ± 0.015∗	0.078 ± 0.007∗•
	Nitrite level (µmol g^{-1} wet weight)	54±5	61±5	305 ± 23∗	98 ± 11∗•
	Vitamin C level (g/ g tissue)	122 ±7	239 ± 25	108±9	213 ± 18∗•

The treated control and diabetic rats received ascorbic acid (1g/l) and cobalt chloride (0.5 mM)-supplemented water. Values are means ± SD of six rats. Significance of difference was assessed by Kruskall-Wallis test p values are shown as ∗$p < 0.05$ vs. control groups; •$p < 0.05$ vs. diabetic group.

Table 2. Comparison of parameters of rat lung with control, diabetic, vitamin C with cobalt treated control and diabetic animals.

Organ	Parameter	Control	Vıtamın C + Cobalt	Dıabetıc	Dıabetıc + Vıtamın C + Cobalt
LUNG	SOD activity (U mg^{-1} protein)	17.3 ± 0.4	18.0 ± 0.4	28.9 ± 0.9 ∗	19.2 ± 0.5∗•
	GSH-Px activity (mol NADPH min^{-1} mg^{-1} protein)	0.28 ± 0.03	0.25 ± 0.03	0.63 ± 0.06 ∗	0. 57 ± 0.04∗
	CAT activity (k $s^{-1}mg^{-1}$ protein)	0.023 ± 0.002	0.024 ± 0.003	0.060 ± 0.004 ∗	0.036 ± 0.004∗•
	MDA (nmol TBARS mg^{-1} protein)	0.12 ± 0.006	0.14 ± 0.006	0.22 ± 0.009∗	0.18 ± 0.005∗
	Nitrite level (µmol g^{-1} wet weight)	35 ± 3	36 ± 4	24±2∗	22±2∗
	Vitamin C level (g/ g tissue)	63 ± 3	81 ± 7	59±5	66±7

The treated control and diabetic rats received ascorbic acid (1g/l) and cobalt chloride (0.5 mM)-supplemented water. Values are means ± SD of six rats. Significance of difference was assessed by Kruskall-Wallis test p values are shown as ∗ $p < 0.05$ vs. control groups; • $p < 0.05$ vs. diabetic group.

weight.

Tissue vitamin C contents were measured in homogenates by the method of Roe and Kuether (1967) (in György and Pearson, 1967) in which the colored complex formed was measured spectrophotometrically. The protein content of the samples was measured by the method of Lowry et al. (1951) with the use of bovine serum albumin as a standard.

All experiments were performed using at least 6 animals in each group and the results were expressed as mean ± standard deviation (SD) . Kruskall-Wallis test was used to compare the means of two groups and analysis of variance was used to compare the other groups. Statistical significance was considered at $p < 0.05$.

RESULTS

The thiobarbituric acid reactive substance (TBARS), nitrite and vitamin C levels and the activity variations of CAT, GSH-Px, SOD in liver, lung, heart and kidney of diabetic rats were investigated at the end of eigth week of treatment. The antioxidant status of liver in various experimental groups are summarized in Table 1. Measurement of free radical scavenging enzymes in diabetic rats showed a general increase in the activities of SOD, GSH-Px, CAT and also in TBARS and nitrite levels compared to respective controls (healty rats). However, the vitamin C level in diabetic animals were decreased.

Table 2 depicts the antioxidant status and the levels of TBARS and nitrite at the end of eight week after STZ injection. As seen in Table 2, SOD, GSH-Px and CAT activities in diabetic rat lung was significantly increased compared to healty groups. In treated groups, SOD and CAT activities decreased significantly compared to diabetic, whereas nitrite levels decreased compared to controls. Vitamin C levels didn't change in diabetic and treated tissue.

Oral administration of vitamin C with cobalt to diabetic rat heart approximatelly 20% normalized the 62% of increased SOD activities at the end of eigth week. A similar pattern was observed in GSH-Px and CAT activities an average restore of 32 and 37%, respectively occur in treated groups. An average healty TBARS value in the heart was 0.060 ± 0.0019 nmol MDA/mg protein. There was an increase in the levels of TBARS of diabetic rats at 8^{th} (2.7 fold) weeks as compared to healty groups. The treatment significantly decreased (30% restore, $p < 0.05$) the increased TBARS levels as compared to diabetics. The change in nitrite and vitamin C levels are

Table 3. Comparison of parameters of rat heart with control, diabetic, vitamin C with cobalt treated control and diabetic animals.

Organ	Parameter	Control	Vıtamın C + Cobalt	Dıabetıc	Dıabetıc + Vıtamın C + Cobalt
HEART	SOD activity (U mg^{-1} protein)	76 ± 2.3	78 ± 2.6	122 ± 4.3∗	98 ± 3.2∗•
	GSH-Px activity (mol NADPH min^{-1} mg^{-1} protein)	0.16 ± 0.011	0.17 ± 0.011	0.37 ± 0.019∗	0.25 ± 0.05∗
	CAT activity (k $s^{-1}mg^{-1}$ protein)	0.023 ± 0.002	0.024 ± 0.003	0.057 ± 0.004∗	0.036 ± 0.004∗•
	MDA (nmol TBARS mg^{-1} protein)	0.057 ± 0.0033	0.063 ± 0.0036	0.106 ± 0.012∗	0.075 ± 0.006∗•
	Nitrite level (µmol g^{-1} wet weight)	39 ± 2	38±3	105 ± 9∗	78 ± 5∗•
	Vitamin C level (g/ g tissue)	65 ± 5	105±7	68±6	98 ± 7∗•

The treated control and diabetic rats received ascorbic acid (1g/l) and cobalt chloride (0.5 mM)-supplemented water. Values are means ± SD of six rats. Significance of difference was assessed by Kruskall-Wallis test p values are shown as ∗ $p < 0.05$ vs. control groups; •$p < 0.05$ vs. diabetic group.

Table 4. Comparison of parameters of rat kidney with control, diabetic, vitamin C with cobalt treated control and diabetic animals.

Organ	Parameter	Control	Vıtamın C + Cobalt	Dıabetıc	Dıabetıc + Vıtamın C + Cobalt
KIDNEY	SOD activity (U mg^{-1} protein)	146±6	157±6	222 ± 11∗	186 ± 7∗•
	GSH-Px activity (mol NADPH min^{-1} mg^{-1} protein)	0.14 ± 0.013	0.16 ± 0.015	0.29 ± 0.019∗	0.25 ± 0.02∗
	CAT activity (k $s^{-1}mg^{-1}$ protein)	0.031 ± 0.0028	0.033 ± 0.004	0.062 ± 0.0035 ∗	0.040 ± 0.003 •
	MDA (nmol TBARS mg^{-1} protein)	0.068 ± 0.005	0.071 ± 0.005	0.156 ± 0.020∗	0.102 ± 0.015∗•
	Nitrite level (µmol g^{-1} wet weight)	34±3	35±3	156 ± 14∗	61 ± 5∗•
	Vitamin C level (g/ g tissue)	69±4	98±7	59±3∗	89 ± 6∗•

The treated control and diabetic rats received ascorbic acid (1g/l) and cobalt chloride (0.5 mM)-supplemented water. Values are means ± SD of six rats. Significance of difference was assessed by Kruskall-Wallis test p values are shown as ∗ $p < 0.05$ vs. control groups; • $p < 0.05$ vs. diabetic group.

also shown in Table 3. The levels of nitrite in treated group decreased 26% compared to diabetic groups. The level of vitamin C in treated group 44 % increased with respect to controls.

In the diabetic rat kidney 46% increase in SOD activity was observed compared to healty group (Table 4). Ascorbic acid with cobalt administration decreased SOD activities from 222 ± 11 U mg^{-1} protein to 186 ± 7 U mg^{-1} protein. As can be seen from Table 4, in the diabetic group the kidney GSH-Px and CAT activities were increased 93% compared to healty animals. Oral administration of vit C and $CoCl_2$ to diabetic rats restored the 15 and 35% of kidney GSH-Px and CAT activities within eigth week, respectively. The TBARS and nitrite levels were 2.2 and 4.5 fold increased, respectively in diabetic animals compared to healty groups. On the other hand, TBARS and nitrite levels were significantly decreased by approximately 35% and 61% respectively in kidney tissue of ascorbic acid and cobalt treated diabetic rats at the end of week eight. Mean kidney ascorbic acid levels (± SD) in the healty, diabetic, treated healty rats and treated diabetics are also shown in Table 4. Kidney vitamin C levels were slightly lower in diabetics compared to respective healty rats (15% decreased in diabetics compared to its control; 10% decreased in treated groups compared treated controls).

DISCUSSION

In the present study we have shown that there is an decrease in the increasing activities of SOD, CAT, the level of lipid peroxidation and nitrite by vitamin C with cobalt administration in the liver, lung, heart and kidney tissues in diabetes.

The all antioxidant enzyme activities and the lipid peroxidation and nitrite levels increased in the diabetic tissues suggesting an increase in reactive oxygen species (ROS) with time. In diabetes, increase in ROS through autoxidation or protein glycation could cause membrane lipid peroxidation and cellular damage and can affect other macromolecules. The increase in antioxidant enzyme activities could be due to induction by increased superoxide anion and hydrogen peroxide. Asayama et al. (1991) indicated that hypoinsulinemia increases the activity of fatty acyl-CoA oxidase which

initiates - oxidation of fatty acids resulting in increased production of hydrogen peroxide. In the present study, also we showed that the hydrogen peroxide related enzyme activities were high. Hydrogen peroxide can also react with superoxide anion to form hydroxyl radical and result in increased lipid peroxidation so increased in TBARS levels (Halliwell and Chirico, 1993). The results showed that the vitamin C with cobalt administration does not increase the healty group antioxidant enzyme activities. The increase may be prooxidant role of vitamin C with cobalt chloride. Because of vitamin C important role as an antioxidant it seems parodoxical that under certain conditions ascorbate could be promote the generation of ROS (Carr and Frei, 1999). In the presence of oxygen and divalent cations (especially iron and copper) may be in the presence of cobalt, ascorbate itself converted to an ascorbate free radical, so autoxidation may occur. On the other hand, a large number of reports suggest that ascorbic acid metabolism is altered in diabetics (Yue et al., 1989; Siman and Eriksson, 1997; Harding et al., 2008) . Vitamin C has been reported to be present normally in millimolar concentrations in humans and animals and is known to be one of the major water soluble physiological antioxidants in the aqueous compartment of cells (Chen, et al. 1983; Moordian, 1987; Padh, 1991). Ascorbate and glucose have a common transport mechanism (Cunningham, 1988; Behrens and Madere, 1991) and the depletion of tissue stores of ascorbic acid by chronic hyperglycemia in STZ-induced diabetes has been reported (Yew, 1983) . In the present study, the combination of ascorbic acid and cobalt seems to act in a synergistic manner allows significant reduction in the elevated antioxidant enzyme activities and TBARS, nitrite levels at the end of eigth week after treatment.

The biological importance and a number of studies are contiuing to examine the role of nitric oxide in diabetes (Ignarro et al., 1987; Ischiropoulos et al., 1992; Moncada and Higgs, 1993; Wink et al., 1999; Satoh et al., 2005; Macarthur et al., 2008; Török, 2008). Deficient or excess NO has been related to several pathological conditions. It has been reported that, NO formation may play a role in the destruction of the pancreatic cells during the development of diabetes (Corbett et. al., 1993). NO may protect against reactive oxygen species from the tissue (Wink et al., 1999). In the present study we shown that there is an increase in the level of nitrite in liver, heart almost in kidney, decreased in lung during diabetes. Increased glucose concentration in diabetes induces elevation in intracellular levels of diacylglycerol activates protein kinase C, which has been shown to activate nitric oxide synthases. Studies on patients with diabetes mellitus, a disorder associated with hyperglycemia and accompanying complications, have revealed oxidative stress loads. It has been also indicated that, nitric oxide is an important mediator, governing a range of physiological functions in animals, from controlling smooth muscle tone in the cardiovascular, gastrointestinal, respiratory and genitourinary systems, to neurotransmission and a role in immune function and inflamation (Singh and Evans, 1997).

The levels of vitamin C in diabetic rat kidney after two, four and six weeks of diabetes induction results in decreased as compared to healty. This decrease in vitamin C level could be due to decrease transport of ascorbic asid as glucose which share the same transport system (Moordian, 1987; Sinclair et al., 1994) and in diabetes hyperglycemia could be responsible for decreased transport of vitamin C. Also the decrease in vitamin C levels may be due to its consumption during the free radical scavenging process.

In conclusion, the defense system against oxidative stress in liver, lung, heart and kidney quickly responds to streptozotocin treatment, resulting in an increase in SOD, CAT, GSH -Px enzyme activities, TBARS and nitrite levels. The present investigation indicates that vitamin C with cobalt administration is effective in controlling the antioxidative system of diabetic rats at the end of eigth week. Our data have shown that peroxidative damage of liver, lung, heart and kidney are manifested in progression of diabetes, with corresponding changes in the antioxidative state of these tissues. Oral administration of vitamin C with cobalt decreased SOD activity values and the considerably decreased activities of CAT and GSH-Px, probably indicates decreased endogenous hydrogen peroxide production. Unlike insulin, in these doses vitamin C with a combination of cobalt fails to completely normalize the antioxidant enzyme activities in these tissues which are crucial importance for oxidative stress. On the other hand, cobalt is essential nutritional trace element and has therapeutic value in pharmacological doses, but is toxic in excess and causes stimulation of H_2O_2 production and lipid peroxidation (Hanna et al., 1992).

In view of the various hyperglycemia lowering effects of vitamin C with cobalt chloride, an attempt was made to study the efficacy of this treatment in controlling the altered antioxidant status of diabetic rat liver, heart, kidney and partially lung tissues. As becoming the answer of the topic we can say that further molecular information from clinical studies are necessary to prove the benefits of this supplementation.

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