Effect of different doses of sodium ortho vandate supplementation on DNA and sialic acid in rats

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Abstract

The present study was conducted to investigate the biochemical effect of low and high doses of sodium ortho- vandate supplementation on some blood and organ parameters in rats. The experimental study was carried out on 63 adult male rats weighing 150-200 gm. Rats were randomly divided into three equal groups each one comprised 21 rats and classified as follow: Group I (control group), group II (low dose of sodium ortho-vanadate group received 0.2 mg/ml sodium chloride solution 80 mM) and group III (high dose of sodium ortho-vanadate group received 0.6 mg/ml sodium chloride solution 80 mM) via drinking water. Blood samples and tissue specimens were collected after 2, 4 and 6 weeks from the onset of sodium ortho-vanadate supplementation. The obtained results revealed that a reduction ($P< 0.05$) in serum total sialic acid (TSA) concentration and the percentage of liver DNA fragmentation were observed after 4 and 6 weeks of low dose of sodium ortho-vanadate, 0.2 mg/ml (VLD) administration as compared with control. Meanwhile a marked increase ($P< 0.05$) in TSA and percentage of liver, heart DNA fragmentation were recorded after administration of sodium ortho-vandate at high dose, 0.6 mg/ml (VHD) all over the period of the experiment. In addition, an elevation in heart and liver DNA content and reduction in the percentage of heart DNA fragmentation were observed after 6 weeks in VLD- treated group. From the obtained results it could be concluded that, low dose of sodium ortho-vanadate has antioxidant and anti-apoptotic effect, while high dose of sodium ortho-vanadate has pro-oxidant and apoptotic effect.

Key words: sodium ortho-vanadate, dose, oxidative stress, DNA, sialic acid, rats.

Introduction

Vanadium is one of heavy metals that considered being a micronutrient and is included in the list of 40 essential micronutrients required in small amounts for normal metabolism (*Ray et al, 2005).* Vanadium is commercially available as inorganic and organic compounds. Sodium ortho- vanadate (Na_3VO_4, SOV) is considered as inorganic compound (*Scior et al, 2005).* Food is the major source of exposure to vanadium for general population. Many cereals, fishes, fresh fruits and vegetables contain this element more than 40 mg per gram of food (*Badmaev et al, 1999).* It is likely important to establish that vanadium is capable of exhibiting some unique beneficial effects at cellular and sub-cellular levels at very low doses *(Bishayee and Chatterjee, 1995).* It is well established that, vanadium exhibits anti-

oxidant activity at low doses *(Li et al, 2009)*. Administration of ammonium mono vanadate (0.5 ppm) for 30 days before tumor implantation in rats prevents mammary carcinogenesis *(Ray et al, 2005)*. On the other hand, a pro-oxidant potential was shown at high dose *(Ścibior et al, 2009)*. Vanadium can induce in vivo hepatotoxicity *(Rattner et al, 2006)* and cardiotoxicity *(Aureliano et al, 2002)*. Additionally, administration of SOV (0.6 mg/ml of drinking water) has been proved to be toxic not only to diabetic but also to normal rats as evidenced from the observations on the blood urea, plasma and liver aspartate transaminase and alanine transaminase *(Preet et al, 2005)*.

Material and Methods

Sixty three adult male Sprague Dawley rats weighing 150-200 g were obtained from Laboratory Research Center in Faculty of Veterinary Medicine (Zagazig University). They were housed in metal cages, fed on a balanced ration and water was supplied ad libitum. Rats were kept 2 weeks for acclimation before beginning of the experiment. Rats were randomly divided in to 3 equal groups each had 21 rats as follow; Group I served as control, Group II received low dose of sodium orthovanadate (0.2 mg/ml sodium chloride solution 80 mM) and Group III received high dose of sodium orthovanadate (0.6 mg/ml sodium chloride solution 80 mM) via drinking water.

The animals were decapitated, and then blood, liver and heart samples were collected from all animal groups (control and experiment group) after 2, 4 and 6 weeks from the onset of SOV supplementation. Blood samples were collected for serum separation from the medial canthus of the eye for determination of total sialic acid (TSA) according to the method described by *(Warren, 1959; Aminoff, 1961 and Denny et al, 1983)*. Moreover, liver and heart were dissected, and then washed with normal saline for determination of DNA content *(Laird et al, 1991;* Perandones et al, 1993; Stamm and Berka, 2006) and percentage of DNA fragmentation by using diphenylamine (DPA) assay (Perandones et al, 1993; Bagachi et al, 2000; Savitha and Panneersel, 2007).

All data were subjected to statistical analysis by F-test and LSD (Snedecor and Cochran, 1982).

Results and Discussion

The data in Table 1 revealed a decrease in serum TSA observed in VLD- treated group after 4 and 6 weeks of experiment. This may be attributed to vanadium at low dose has antioxidant properties *(Preet et al, 2005 and Li et al, 2009)*. Thus vanadium protects sialic acid (S.A) in cell membrane against attack by reactive oxygen species (ROS) which induced shedding of aberrant S.A-containing cell surface glycoconjugates *(Goswami and Koner, 2002)*. While, an increase in serum TSA was observed in VHD- treated group during the whole period of the experiment. This may be due to ROS is produced during oxidation-reduction of vanadium leading to an increase in the activity of sialidase enzyme which cleaves SA as the terminal sugar residues on oligosaccharides in glycoproteins of cell membrane resulting in shedding of

SA into circulation (Shi and Dalal, 1992; Goswami and Koner, 2002 and Soares et al, 2008). Another suggestion proposed that tissue injury caused by ROS can stimulate local cytokine secretion from macrophages (Schulze et al, 1997 and Crook et al, 2001). This induces an acute phase inflammatory response with an increase in the output of acute phase reactants from the liver into the general circulation which actually are glycoproteins have SA as the terminal sugar on their oligosaccharide chain (Bendigar et al, 2006 and Patil et al, 2007).

Table (1). Effect of different doses of sodium ortho-vandate on serum total sialic acid (TSA) concentration in rats (mg/dl).

Durations / Animal groups	2 weeks	4 weeks	6 weeks
Control	55.53 ± 2.13^{aA}	58.67 ± 2.66^{aA}	60.55 ± 2.57^{aA}
VLD	52.70 ± 1.79^{aA}	42.35 ± 1.49^{bB}	31.06 ± 2.00^{bC}
VHD	69.65 ± 3.24^{bA}	92.23 ± 2.71^{cB}	116.70 ± 3.37^{cC}

Values represent mean ± SE. Mean values with different small superscripts in the same column and capital letters in the same raw are significantly different. *SE*= standard error. *VLD*= vanadium low dose (0.2 mg/ml) group, *VHD*= vanadium low dose (0.6 mg/ml) group.

Apoptosis is a programmed cell death involves the regulated action of catabolic enzymes in response to any mild injury or moderate oxidative stress while necrosis has been considered merely as an accidental uncontrolled form of cell death in response to more severe forms of the same types of injury *(Saito et al, 2001 and Kroemer et al, 2009)*. In this work, VLD-treated group shows a pronounced increase in heart and liver DNA content after 6 weeks (*table 2),* while reduction in percentage of DNA fragmentation (*table 3*) in heart after 6 weeks and in liver after 4& 6 weeks post administration.

Table (2) Effect of different doses of sodium ortho-vandate on liver and heart DNA content in rats.

Parameters / Animal groups	Liver DNA content (µg/ mg tissue)			Heart DNA content (µg/ mg tissue)		
	2weeks	4weeks	6weeks	2weeks	4weeks	6weeks
Control	4.08 ± 0.05^{aA}	4.06 ± 0.05^{aA}	4.02 ± 0.04^{aA}	2.01 ± 0.04^{aA}	2.01 ± 0.02^{aA}	1.99 ± 0.02^{aA}
VLD	4.09 ± 0.03^{aA}	4.10 ± 0.05^{aA}	4.23 ± 0.03^{bB}	2.02 ± 0.02^{aA}	2.05 ± 0.03^{aA}	2.14 ± 0.01^{bB}
VHD	3.61 ± 0.08^{bA}	2.55 ± 0.03^{bB}	1.76 ± 0.03^{cC}	1.83 ± 0.03^{bA}	1.51 ± 0.03^{bB}	1.12 ± 0.03^{cC}

Values represent mean ± SE. Mean values with different small superscripts in the same column and capital letters in the same raw are significantly different. *SE*= standard error. *VLD*= vanadium low dose (0.2 mg/ml) group, *VHD*= vanadium low dose (0.6 mg/ml) group.

This may be attributed to the antioxidant action of low dose of vanadium as it increases glutathione *(Yanardag and Tunali, 2006; N-Dar et al, 2007)*. GSH detoxifies toxic metabo-

lites and its depletion can induce both forms of cell death; apoptosis and necrosis *(Lauterburg, 2002 and Higuchi, 2004)*. Similarly, vanadium increases catalase and super oxide dismutase (*Ramachandran et al, 2004 and Tas et al, 2006*) which are able to inhibit the 8- Hydroxyl-2'deoxyguanosine (8-OH-dG) formation through inhibition of •OH radical formation (*Strasser et al, 2000*). Additionally, *Bhuiyan et al, 2008* revealed that, the ability of vanadium compounds to activate protein kinase B (PKB; c-Akt) signaling pathways are responsible for their ability to modulate cardiovascular functions and is probably beneficial as a cardioprotective drug in subjects undergoing reperfusion therapy following myocardial infarction. Moreover, low dose of vanadium induced suppression of DNA double-strand breaks due to the capability of the post replication repair and excision repair activity *(Chattopadhyay et al, 2005)*.

On the other hand, rats in VHD-treated group suffered from lower DNA content and higher percentage of DNA fragmentation in liver and heart after 2, 4 and 6weeks post administration. These results are in agreement with those of *(Badria et al, 2009)* who reported that, the diminution of cell DNA content is a typical feature of cells undergoing apoptosis and is considered as a new fibrosis index biomarker in which DNA content increases with increasing cell division and decreases with increasing cell death. It is reported that the plausible hypothesis for the cytotoxicity of vanadium may be due to one-electron reduction of pentavalent vanadium to tetravalent vanadium that occur via glutathione- dependent mechanism resulting in reduction in GSH content which is accompanied by an increase in TNF-α production involved in apoptosis *(Lu et al, 2001 and Han et al, 2006)*. This resulted in the formation of $O_2^{\cdot-}$ and H_2O_2 which in turn leads to .OH radical formation that attack DNA causing 8- hydroxyl-2'deoxyguanosine (8-OH-dG) formation and substantial DNA fragmentation *(Higuchi, 2004; Savitha and Panneerselvam, 2007)*. Moreover, *Higuchi and Yoshimoto, (2004)* reported that, apoptosis can be converted into necrosis accompanied with a reduction of internucleosomal DNA fragmentation which occur in apoptosis. This change may be dependent on the intensity of oxidative stress and lipid peroxidation. Poly unsaturated fatty acids (PUFA) such as arachidonic acid cause necrosis at high concentrations under GSH depletion, promoting giant DNA fragmentation of chromatin. Additionally, mitochondrial GSH depletion leads to accumulation of ROS that lowers the ATP synthesis which is required for repairing DNA damage resulting in cell injury and cell death *(Osley et al, 2007; Savitha and Panneerselvam, 2007)*.

Table (3) Effect of different doses of sodium ortho-vandate on liver and heart Percentage of DNA fragmentation in rats.

Parameters / Animal groups	Liver DNA Fragmentation (%)			Heart DNA Fragmentation (%)		
	2weeks	4weeks	6weeks	2weeks	4weeks	6weeks
Control	7.44± 0.31^{aA}	7.59± 0.31^{aA}	7.93± 0.12^{aA}	5.62± 0.14^{aA}	5.64± 0.12^{aA}	5.76± 0.20^{aA}
VLD	7.15± 0.26^{aA}	6.67± 0.27^{bA}	3.71± 0.16^{bB}	5.64± 0.27^{aA}	5.25± 0.26^{aA}	3.38± 0.11^{bB}
VHD	8.26± 0.57^{bA}	10.44± 0.13^{cB}	14.73± 0.16^{cC}	6.47± 0.22^{bA}	8.09± 0.15^{bB}	11.31± 0.26^{cC}

Values represent mean ± SE. Mean values with different small superscripts in the same column and capital letters in the same raw are significantly different. *SE*= standard error. *VLD*= vanadium low dose (0.2 mg/ml) group, *VHD*= vanadium low dose (0.6 mg/ml) group.

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تاثيرالجرعات المختلفة من الصوديوم أورثو فاندات على الحمض النووى الداى أوكسى الريبوزى وحمض السياليك في الفئران

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الملخص العربى

يهدف البحث الى دِراسَة التأثير العلاجّي (٠,٢ مجم/ ميليلتر) للجرعةِ المنخفضةِ و التأثير السامّ (٠,٦ مجم/ ميليلتر) للجرعةِ العاليةِ لصوديوم أورثو فاندات في الفئران. أجريت الدراسة على ٦٣فأر أبيض ذكر يزن١٥٠ – ٢٠٠جم قسمت الى ثلاثة مجموعات كالتالى:- (مجموعة ضابطة – صوديوم أورثو فاندات (٠,٢ مجم/ ميليلتر مِنْ ماء الشربِ)- صوديوم أورثو فاندات (٠,٦ مجم/ ميليلتر مِنْ ماء الشربِ). سجلت النتائج زيادة فى محتوى الحمض النووى الداى أوكسى الريبوزى بالكبد والقلبِ بعد ٦ أسابيع من اعطاء الجرعةِ المنخفضةِ من صوديوم أورثو فاندات بالمقارنة بالمجموعة الضابطة. بينما وجد نقصُ فى تلك المعاملات طول فترة التجربة من اعطاء الجرعةِ العالية بالمقارنة بباقى المجموعات. كما وجد نقصُ فى مستوى حمض السياليك بالمصلَ و نسبة تكسير الحمض النووى الداى أوكسى الريبوزى بالكبد بشكل ملحوظ بعد ٤&٦ أسابيع و نسبة تكسير الحمض النووى الداى أوكسى الريبوزى بالقلبِ بعد ٦ أسابيع من اعطاء الجرعةِ المنخفضةِ بالمقارنة بالمجموعة الضابطة مع زيادة فى تلك المعاملات طول فترة التجربة من إعطاء الجرعةِ العالية بالمقارنة بباقى المجموعات. أثبتت الدراسة أن للجرعةِ المنخفضةِ من الصوديوم أورثو فاندات دوراً هاماً كمضاد للأكسدة بينما كان للجرعة العالية تأثير إجهاد تأكسدي و سامّ في الدمِّ والكبدِ والقلبِ. لهذا كلا التاثيران يعتمدان على الوقت و الجرعةِ.