Potential protective role of *β*-cryptoxanthin against testicular oxidative stress induced by vinyl cyanide exposure in male rats

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ABSTRACT: Vinyl cyanide (VCN) is an aliphatic nitrile product which is extensively used in various synthetic chemical industries. VCN is known to exert toxic actions to human beings as well as experimental animals. The present study was designed to examine the ability of β-cryptoxanthin, a naturally occurring antioxidant, to attenuate VCN-induced testicular toxicity in adult albino rats. Daily oral administration of VCN at a dose level of 30 mg/kg b.w. (7.2mg/ animal) to male rats for a period of 5 days significantly decreased serum and testicular glutathione (GSH) content and glutathione-S-transferase (GST) activity. While, VCN induced lipid peroxidation as indicated by markedly increased of malondialdehyde (MDA). Compared to VCN-treated animals, pretreatment with β-cryptoxanthin and its co-administration with VCN once daily at a dose of 40 mg/kg b.w. (9.6mg/ animal) for 30 days mitigates serum and testicular GSH content, GST activity and MDA level. In conclusion, the present results clearly demonstrate the protective role of β-cryptoxanthin against VCN-induced oxidative stress in the rat testis.

Keywords: Vinyl cyanide; β-cryptoxanthin; Oxidative stress; Testes.

INTRODUCTION

Vinyl cyanide (C_3H_3N, VCN), Also known as acrylonitrile, a highly reactive compound having an active vinyle and cyanide groups, has been widely used in industry for production of plastics, elastomers, and synthetic fibres and as an intermediate in the synthesis of industrial chemicals and pharmaceuticals (IARC, 1979). It is also used in the manufacture of soft prosthesis material (Parker and Braden, 1990), coating membranes for Langerhans islets implants (Kessler *et al*., 1992) and high permeable dialysis tubing (Ward *et al*., 1993).

Human exposure to VCN could potentially occur during the manufacturing process, end product usage and transportation. Further, such exposure can also be possible in the general population through cigarette smoke and *via* contamination of drinking water (Byrd *et al*., 1990). VCN demonstrated acute toxicity in testes of rats, mice, rabbits and guinea pigs having a high acute toxicity from inhalation and a high to extreme acute toxicity from oral or dermal exposure (Their *et al*., 2000). VCN is teratogenic in laboratory animals (rat and hamster) at high doses when maternal toxicity has been already manifested. VCN has been demonstrated to induce embryotoxic effects in rat (Saillenfait and Sabate, 2000). VCN-induced embryotoxic and teratogenic effects have also been found in VCN-exposed workers (Wu *et al*., 1995). According

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to environmental teratologic epidemiological study in inhabitants living in the surrounding region of an acrylonitrile factory, three congenital abnormalities (pectus excavatum, undescended testis and clubfoot) in 46,326 infants showed significant time-space clusters in the study region. There was a decrease in risk of undescended testis with increasing distance from the acrylonitrile factory (Czeizel *et al*., 1999). Therefore, women not professionally exposed would appear to be at risk of teratogenic effects due to VCN toxicity.

VCN is rapidly absorbed and distributed to all major tissues in animals. Previous studies with 14C have shown that VCN covalently binds to thiol group of proteins (Ahmed *et al*., 1982) and tissue macromolecules and nucleic acids (Pilon *et al*., 1988). Therefore, estimation of free radical generation and antioxidant defence has become an important aspect of investigation in mammals. Carotenoids (β-cryptoxanthin or what is known as β-carotene) are naturally occurring antioxidants that play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. The antioxidant function of these micro-nutrients could, at least in part, enhance the immunity by maintaining the functional and structural integrity of important immune cells (Chew, 1995; El-Demerdash *et al*., 2004).

β-cryptoxanthin, aside from being a major source of vitamin A (retinol), an essential vitamin for spermatogenesis to proceed, has been reported to be a potent free radical quencher, singlet oxygen scavenger and lipid antioxidant (El-Missiry and Shalaby, 2000). Furthermore, Burton (1989) focused on the ability of β-cryptoxanthin to function as a chain-breaking antioxidant in a lipid environment at physiological oxygen partial pressures that are considered most likely in mammalian cells. Therefore, the aim of the current study was to investigate the efficacy of β-cryptoxanthin on VCN-induced functional and structural alterations related to oxidative stress in the testes of rats.

MATERIALS AND METHODS

Chemicals:

Vinyl cyanide (VCN) and β-cryptoxanthin were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and given by oral gavage at dose levels of 30 mg/kg b.w. (Takano *et a*l., 2010) and 40mg/kg b.w. (Sadir *et a*l., 2007), respectively. All other chemicals and solvent used were of highest available commercial grade.

Experimental animals:

Forty male Sprague–Dawely rats, each weighing 240 ± 10 g. The animals were housed in stainless steel cages after grouping in batches of five under standard animal house conditions of relative humidity (55 ± 5%), temperature (25 ± 2 ˚C) and a 12 hr light/12 hr dark cycle. Rats were allowed free access to standard commercial feed and tap water and were acclimatized to laboratory conditions for a period of one week before the onset of experimentation.

Experimental protocol:

Animals were allocated to four groups each of ten rats as follows:

Group I: (Control) pre-treated with corn oil (2 ml/kg b.w.) once daily for 25 days and treatment continued with distilled water (2 ml/kg b.w.) once daily for additional 5 days i.e. from day 26 to day 30 of the experimental period of 30 days.

Group II: (VCN group) pre-treated with corn oil (2 ml/kg b.w.) once daily for 25 days and treatment continued with VCN in a dose of 30 mg in 2ml distilled water per kg b.w. (7.2 mg/ animal) once daily for additional 5 days.

Group III: (β-cryptoxanthin group) pre-treated with β-cryptoxanthin in a dose of 40 mg in 2 ml corn oil per kg b.w. (9.6mg/ animal) once daily for 25 days and treatment continued with distilled water (2 ml/kg b.w.) for additional 5 days.

Group IV: (β-cryptoxanthin and VCN group) pre-treated with β-cryptoxanthin (40 mg/kg b.w.) for 25 days and treatment continued with VCN (30 mg/kg b.w) for additional 5 days.

At the end of the experimental period, the tested animal groups were sacrificed after 24 hr. of the last dose of different administrations and their blood were collected, by carotid bleeding, in centrifuge tubes and serum was obtained from the blood after centrifugation at 3000 rpm for 10 min. The testes were immediately excised and stored at -20°C until analysis studies.

Methods of analysis:

Glutathione (GSH) was spectrophotometrically assayed in the serum and testes by the method of Sedlak and Lindsay (1968). Glutathione-S-transferase (GST) was assayed in serum and testes by the method of Habig et al. (1974). malondialdehyde (MDA) was determined in serum and testes by using the method of Mihara and Uchiyama (1978).

Statistical analysis:

Statistical analyses of the resulted data were done using InStat version 2.0 (Graph Pad, ISI, Philadelphia, PA, USA, 1993) computer software. The results were expressed as means (±SE).

Multiple comparisons were done using one-way ANOVA followed by Tukey-Kramer as a post-ANOVA test. Statistical significance was accepted at $P < 0.001$, $P < 0.01$, $P < 0.05$.

RESULTS

Analysis studies:

VCN exerts a significant ($P<0.001$) decrease in serum and testis GSH content and GST activity, while the level of MDA in serum and testis significantly ($P<0.001$) elevated as compared to the control values. β-cryptoxanthin treatment significantly ameliorated the VCN-induced decrease in these parameters **(Tables 1, 2 and 3)**.

Table 1: The effect of vinyl cyanide (30 mg/kg b.w.) and/or β-cryptoxanthin (40 mg/kg b.w.) on serum and testicular GSH content of male albino rats.

groups	serum GSH mmol /l	testicular GSH mmol /g
Control	4.23± 0.08	3.93± 0.12
VCN	1.41± 0.12 (-66.67%) a**	1.51± 0.12 (-61.58%) a**
β-cryptoxanthin	4.79± 0.13 (13.24%) a b**	4.30± 0.07 (9.41%) b**
β-cryptoxanthin + VCN	3.37± 0.16 (-20.33%) a**b**c**	3.28± 0.15 (-16.54%) a*b**c**

- Data are expressed as means ± SE (n = 10 in each group).

- Values between parentheses are the difference % of each parameter with respect to control value.

a: Significant change at $P < 0.05$ with respect to control group.

b: Significant change at $P < 0.05$ with respect to VCN-group.

c: Significant change at $P < 0.05$ with respect to β-cryptoxanthin -group.

**Very high significant change exists at $P < 0.001$.

Table 2: The effect of vinyl cyanide (30 mg/kg b.w.) and/or β-cryptoxanthin (40 mg/kg b.w.) on serum and testicular GST activity of male albino rats.

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groups	serum GST mmol /l	testicular GST mmol /g
Control	4.49± 0.11	5.05± 0.16
VCN	2.26 ± 0.05 (- 49.67%) a**	2.46± 0.09 (-51.29%) a**
β-cryptoxanthin	4.83± 0.07 (7.57%) ab**	5.78± 0.58 (14.46%) a**b**
β-cryptoxanthin + VCN	3.49 ± 0.08 (-22.27%) a**b**c**	3.39 ± 0.10 (-32.87%) a**b**c**

Data are expressed as means ± SE (n = 10 in each group).
- Values between parentheses are the difference % of each parameter with respect to control value.
a: Significant change at $P < 0.05$ with respect to control group.
b: Significant change at $P < 0.05$ with respect to VCN-group.
c: Significant change at $P < 0.05$ with respect to β-cryptoxanthin -group.
**Very high significant change exists at $P < 0.001$.

Table 3: The effect of vinyl cyanide (30 mg/kg b.w.) and/or β-cryptoxanthin (40 mg/kg b.w.) on serum and testicular MDA level of male albino rats.

groups	serum MDA nmol /ml	testicular MDA nmol /g
Control	37.78 ± 0.07	17.22± 0.43
VCN	104.67 ± 0.09 (177.05%) a**	43.35± 1.06 (151.74%) a**
β-cryptoxanthin	31.53 ± 0.09 (-16.54%) a**b**	18.13± 0.58 (5.28%) b**
β-cryptoxanthin + VCN	57.42 ± 0.12 (51.99%) a**b**c**	30.91± 0.23 (79.50%) a**b**c**

- Data are expressed as means ± SE (n = 10 in each group).
- Values between parentheses are the difference % of each parameter with respect to control value.
a: Significant change at $P < 0.05$ with respect to control group.
b: Significant change at $P < 0.05$ with respect to VCN-group.
c: Significant change at $P < 0.05$ with respect to β-cryptoxanthin -group.
**Very high significant change exists at $P < 0.001$.

DISCUSSION

The present results demonstrated that VCN is capable of inducing oxidative stress in testes of rats as indicated by significant depletion of both enzymatic (GST) and non-enzymatic (GSH) antioxidant defence system. Furthermore, VCN significantly enhanced lipid peroxidation, as assessed by monitoring MDA production. These findings are in harmony with the known ability of VCN to induce oxidative stress *in vivo* and in astrocytes *in vitro* (Jiang *et al*., 1998; Kamendulis *et al*., 1999). Also, in the same line, the study of Abdel-Wahab *et al*. (2003) who showed that i.p. injection of structurally related compounds such as dibromoacetonitrile (DBAN) in mice significantly decreases the testicular content of GSH and increases the content of MDA.

VCN is metabolized *via* 2 major pathways (Burka *et al*., 1994; Sumner *et al*., 1999). The first pathway entails the direct conjugation of parent VCN with reduced glutathione (GSH). Subsequent degradation of this metabolite leads to the formation and urinary excretion of N-acetyl-S (2-cyanoethyl) cysteine (Fennell *et al*., 1991; Sumner *et al*., 1999). The second pathway

involves epioxidation of VCN *via* cytochrome P450 2E1 (CYP 2E1) leading to the formation of the epoxide intermediate, 2-cyanoethylene oxide (CEO), a reactive and a relatively long-lived epoxide (Kedderis *et al*., 1995). Subsequent metabolism of CEO occurs *via* conjugation with GSH or *via* hydrolysis to yield cyanide (CN^-) and other metabolites. Production of CN^- induces oxidative stress by enhancing hydroperoxide generation and lipid peroxidation. Also, CN^- inhibits enzymes of biological system, the most important being cytochrome oxidase with subsequent cessation of energy production, as indicated by depletion of ATP production. Cessation of energy-dependent pathways could also augment oxidative stress (Esmat *et al*., 2007). Thus, GSH-depleting properties of VCN could be attributed to enzymatic conjugation and/or direct binding with thiol group, which in turn resulted in enhanced lipid peroxidation. Several studies have reported the implication of free radicals such as hydroxyl radial and superoxide anion in the testicular toxicity of VCN (Mashino and Fridovich, 1987; Rashba-step *et al*., 1993; Hodnick *et al*., 1994). The formation of reactive oxygen species ($OH^{\bullet}$,$O^{\bullet}_2{}^-$ and H_2O_2) is done through bioactivation of cyanide-containing compounds (acrylonitrile, dibromoacetonitrile and choloroacetonitrile) by hepatic microsomal enzymes such as cytochrome-P450 or peroxidases leading to liberation of CN ions which interact with certain compounds inducing free radicals formation (Aust *et al*., 1993).

The present study indicates the beneficial effects of β-cryptoxanthin against vinyl cyanide induced testicular toxicity. β-cryptoxanthin treatment mitigates serum and testicular GSH content, GST activity and MDA level. These results are in agreement with those obtained by Hanukoglu (2006) who reported that the antioxidant enzyme activities superoxide dismutase, catalase, and glutathione peroxidase are parallel steroidogenesis and the antioxidant β-cryptoxanthin exerted a protective role on Leydig cell steroidogenesis to produce testosterone; thus it stimulated the development of reproductive organs through the growth of Leydig and Sertoli cells and the promotion of spermatogenesis. A rational mechanism for the protective effects of β-cryptoxanthin is its potential antioxidant activity. Because β-cryptoxanthin is a lipophilic substance, it exerts its action in hydrophobic environment such as the lipid core of membranes. Thus, it is anticipated that natural β-cryptoxanthin, a chain breaking antioxidants, can contribute to protecting cell membranes from lipid peroxidation (Krinsky, 1998). β-cryptoxanthin can function as an effective antioxidant not only against $^1O_2$ but also against lipid peroxidation and the highly destructive, hydroxyl radical $OH^{\bullet}$ that is implicated in many diseases

such as cancer and heart disease (O'Neill and Thurnham, 1998). El-Missiry and Shalaby (2000) indicated that co-treatment with β-cryptoxanthin produces a signifcant reduction in CdCl2-induced increase in lipid peroxidation in rat brain and testis. This finding is paralleled by modulation of SOD and GST activities and GSH content in both tissues. Also, Silva *et al*. (2001) suggested that pretreatment with another carotenoid, bixin reduced the total number of chromosome aberrations and inhibited the increase in lipid peroxidation induced by cisplatin. More recent, Atessahin *et al*. (2006) reported that pre- and post-treatment with lycopene significantly inhibited the increase in MDA and GSH depletion in the testes induced by cisplatin exposure.

In conclusion, this study clearly demonstrated the potential antioxidant benefit of β-cryptoxanthin in relieving VCN-induced oxidative stress in the testes of rats.

الدور الوقائي المحتمل للبيتاكريبتوزانثين ضد الإجهاد التأكسدي في الخصية الناجم عن التعرض لسيانيد الفينيل في ذكور الجرذان

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سيانيد الفينيل هو منتج أليفاتي يستخدم على نطاق واسع في مختلف الصناعات الكيميائية الاصطناعية ومن المعروف أن سيانيد الفينيل له تأثيرات سامة على البشر وكذلك حيوانات التجارب. وقد صُممت هذه الدراسة لفحص قدرة بيتاكريبتوزانثين ، وهو أحد مضادات الأكسدة الطبيعية، على تخفيف الإجهاد التأكسدي الذي يسببه سيانيد الفينيل في الجرذان البيضاء البالغة.

وقد أجريت الدراسة على أربعين من ذكور الجرذان البيضاء البالغة، تزن 10±240 جراما، تم تقسيمها إلى أربع مجموعات (10 جرذان لكل مجموعة). وتمثل المجموعة الأولى (التي أعطيت زيت ذرة وماء مقطر) المجموعة الضابطة. وأعطيت المجموعة الثانية عن طريق الفم جرعات من مركب سيانيد الفينيل تعادل 30 مجم/كجم من وزن الجسم وذلك على مدار الأيام الخمسة الأخيرة من نهاية التجربة. وأعطيت المجموعة الثالثة يوميا عن طريق الفم جرعات من البيتاكربتوزانثين تعادل 40 مجم/كجم من وزن الجسم لمدة 30 يوما، أما المجموعة الرابعة فقد أعطيت البيتاكربتوزانثين وسيانيد الفينيل مثل المجموعتين الثانية و الثالثة وبالجرعات نفسها. وقد تم تشريح مجموعات الجرذان بعد أربعة وعشرين ساعة من إعطاء الجرعات الأخيرة.

وقد أظهرت النتائج أن إعطاء سيانيد الفينيل في المجموعة الثانية قد تسبب في زيادة ملحوظة في تركيز الأكسدة الفوقية للدهون مما يدل على زيادة أكسدة الدهون. إضافة إلى ذلك فقد وجد أن هناك تناقص ملحوظ إحصائيا في محتوى كل من الجلوتاثيون والجلوتاثيون ترانسفيريز في مصل الدم ونسيج الخصية، مما يدل على أن لسيانيد الفينيل القدرة على زيادة الإجهاد التأكسدي وبالتالي التأثير بشكل سلبي على الخصوبة في ذكور الجرذان البيضاء، أما بالنسبة للمجموعة الرابعة التي أعطيت البيتاكربتوزانثين قبل وفي وقت متزامن مع سيانيد الفينيل فقد حدث تحسن ذي دلالة إحصائية في مستوي هذه المعايير.

وبذلك تكون النتائج قد أوضحت أن للبيتاكربتوزانثين دور وقائي في تقليل أضرار الإجهاد التأكسدي في خصى ذكور الجرذان البيضاء الناتجة عن المعاملة بسيانيد الفينيل .

الكلمات المفتاحية: سيانيد الفينيل ; بيتاكربتوزانثين ; إجهاد تأكسدي ; خصى.

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