Does ascorbic acid protect against vanadium neurotoxicity in different regions of rat brain?

Fatum A. El-Shaari, *BSc, MSc,* ***Syed S. Haider,*** *MSc, PhD,* ***Mustafa M. El-Fakhri,*** *PhD,*
Kilani M. Ghawarsha, *PhD.*

ABSTRACT

Objectives: Ascorbic acid is a powerful antioxidant and protects against lipid peroxidation in cerebral membrane. The objective of the study was to investigate protective effects of ascorbic acid on lipid peroxidative damage and perturbation of enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and acetylcholinesterase (AChE) in discrete regions of rat brain after vanadium exposure.

Method: This study was carried out at Al-Arab Medical University, Benghazi, Libya, from 1995 to 1996. To investigate the effect of this vitamin on vanadium neurotoxicity in brain, adult rats were injected with L-ascorbic acid (1.0 g/kg body weight) and elemental vanadium (2.5 mg/kg body weight) alone or concomitantly for 8 consecutive days by the intraperitoneal route.

Results: Vanadium intoxication significantly enhanced the occurrence of lipid peroxidation in discrete regions of rat brain. The increase in lipid peroxidation was inhibited by ascorbic acid treatment. On the other hand, ascorbic acid content was higher, but the activity of G6PDH and AChE was significantly inhibited in discrete regions of rat brain after vanadium neurotoxicity. Interestingly, however, co-administration (L-ascorbic acid + vanadium) reversed the levels of ascorbic acid together with the activity of G6PDH and AChE towards normal values. The neurohistopathology showed that vanadium is involved in an interaction with myelin and deleterious effect of metal resulted in edema and marked vacuolation of white matter or segmental de-myelinantion in the hypothalamus region of rat brain. Ascorbic treatment, however, did not show any effect on histopatholgical changes.

Conclusions: These results suggested that vanadium neurotoxicity initiated lipid peroxidative damage and interferes with enzymes, G6PDH and AChE, in rat brain. These events may lead to sensorimotor dysfunctions and axonal degeneration. On the other hand, ascorbic acid therein reduced the toxicity of vanadium.

Neurosciences 2002; Vol. 7 (4): 278-286

Ascorbic acid is a very important water soluble antioxidant in mammalian tissues.[1] Several investigators have found that ascorbic acid is capable of quickly counteracting toxicity sign of various xenobiotics, such as lead,[2] organophosphates[3] and mercuric chloride.[4] Ascorbic acid may play a useful role in prevention of cancer,[5,6] the common cold;[7] atherosclerosis;[8] and acquired immuno-deficiency syndrome.[9] Ascorbic acid can cross the blood-brain barrier and is highly concentrated in the central nervous system (CNS). Ascorbic acid is generally present in the cerebrospinal fluid (CSF) at levels higher than plasma. From the CSF, it diffuses in the extracellular space of the brain and then is actively transported to the intracellular compartment. Ascorbic acid inhibits lipid peroxide formation in

From the Department of Biochemistry, Al-Arab Medical University, Faculty of Medicine, Benghazi, *Libya*.

Received 17th March 2002. Accepted for publication in final form 26th June 2002.

Address correspondence and reprint request to: Dr. Syed S. Haider, Associate Professor, Aewan-e-Nazar, 4/830, Sir Syed Nagar, Aligarh-202002, Uttar Pradesh, *India*. Tel. +91 (571) 400028. Fax. +91 (571) 702758/406839. E-mail: syedsaleem_haider@rediffmail.com

brain cerebral membrane preparations.[10] There is evidence that ascorbic acid can protect an organism against the toxic action of some metals.[11]

Vanadium is a common trace element and is a potentially toxic environmental pollutant. Vanadium is found in several industries. It has been well established that the exposure to extremely high doses of vanadium in the work place, especially in the petroleum industry, can lead to serious pathological consequences.[12] The CNS, being rich in polyunsaturated fatty acid side chains, high oxygen tension and poor in antioxidant capacity, is vulnerable to free radical damage.[13] Vanadium toxicity in man may lead to tremor and CNS depression.[14] We have demonstrated from this laboratory[15] that exposure of rats to vanadium perturbed the lipid and protein metabolism with concomitant stimulation of occurrence of lipid peroxidation in the discrete brain regions. It was speculated that vanadium damaged the cell membrane via free radical injury. Earlier, we[16] have also investigated that α-tocopherol protected the brain against vanadium-induced free radical injury. Domingo et al[17] studied that ascorbic acid was the most effective substance in preventing vanadium toxic action in mice. In the present investigation, we have examined the influence of ascorbic acid after vanadium intoxication with reference to the concentration of malondialehyde formed, an index of lipid peroxidation, the level of ascorbic acid, status of glucose-6-phosphate dehydrogenase (G6PDH) and acetylcholinesterase (AChE) activity in the discrete areas of rat brain. Furthermore, histopathological studies in the brain tissues were carried out to seek a possibility, if any, of direct interaction of vanadium metal with the myelin sheath.

Methods.

Chemicals. Acetylthiocholine iodide, butylated hydroxytoluene and thiobarbituric acid were purchased from Sigma Chemicals Co., St. Louis, Missouri, United States of America. L-ascorbic acid, sodium metavanadate, tetraethoxypropane and all the other chemicals were purchased from BDH Chemical, England, United Kingdom and were of analytical grade.

Animals. Eighty male Sprague-Dawley rats obtained from the central Animal House of Al-Arab Medical University, Benghazi, Libya, weighing 276g-396g, were employed in this study. The animals were housed 6 per cage with wood chip bedding and maintained on a 12-hour light/dark cycle (dark at 2000 hours). Pellet diet (National Company of Animal Feeds, Benghazi, Libya) and tap water was provided ad libitum.

Treatment. The rats were divided into 4 groups in the following manner: Group 1. Rats were injected with one ml physiological saline/kg body weight for 8 consecutive days by intraperitoneal (i.p.) route. Group 2. Rats were intoxicated with sodium metavanadate (2.5 mg elemental vanadium/kg body weight) in distilled water consecutively for 8 days by i.p. route.[16] Group 3. Served as ascorbic acid controls and was injected with L-ascorbic acid (1.0g ascorbic acid/kg body weight); 10% solution (pH 4.0) in distilled water, consecutively for 8 days by i.p. route. This dose was selected in accordance with a study by Eichbaum et al.[18] Group 4. This group was administered sodium metavanadate (2.5mg elemental vanadium/kg body weight) and L-ascorbic acid (1.0 g/kg) body weight, 10% solution; (pH 4.0) in distilled water, consecutively for 8 days by i.p. route.

Neurochemical investigations. Rats were weighed and sacrificed by decapitation 24 hours after last injection. The brains were excised quickly on glass plate resting over crushed ice, and the hippocampus, hypothalamus, mid-brain and medulla and pons were dissected.[19] The tissues were weighed and processed immediately for the analysis. Each region of the brain was homogenized in chilled 0.15M potassium chloride (KCl) using a glass homogenizer with teflon pestle fitted to motor drive, and volume was adjusted to give a 10% w/v homogenate. Glucose-6-phosphate dehyhdrogenase (EC 1.1.1.49) activity was assayed by utilizing Boehringer Mannheim GmbH Diagnosta, Germany (Cat No. 124672) Kit. The procedure was based upon the method as described by Lohr and Waller,[20] in which the increase in production of nicotinamide adenine dinucleotide phosphate (NADPH) from NADP+ was followed and glucose-6-phosphate was measured spectrophotometrically at 340 nm. Acetylcholinesterase activity was determined by the method as described by Plummer.[21] The assay mixture contained 0.1 M sodium phosphate buffer (pH 8.0), 10 mM 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (158.5 mM/liter), and sample (50 µl) in a final volume of 3 ml. The mercaptan formed as a result of the hydrolysis of the ester then was reacted with an oxidizing agent DTNB which was split into 2 products, one of which (5-thio-2-nitrobenzoate) absorbed at 412 nm. The activity of the enzyme was measured by following the increase in absorbance at 412 nm in a spectrophotometer. And was calculated as litre Moles/min/ml as has been expressed by Plummer.[21] Ascorbic acid was estimated spectrophotometrically according to the method as described by Kyaw.[22] Phosphotungstic acid used in this method served as protein precipitant and ascorbic acid extractant. The method used for determination of lipid peroxidation was modified in order to make it more sensitive than earlier adopted methods. Lipid peroxidation in 10% (w/v) tissue homogenate prepared in 0.15 M KCl was quantitated according to the method of Ohkawa et al.[23] The method was based on the formation of a red chromophore, which was absorbed at 532 nm, following the reaction of thiobarbituric acid (TBA)

with malondialdehyde and other breakdown products of peroxidized lipids. The method consisted of the addition of the following to 30 ml scintillation vials: 0.2 ml of a 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 ml of 20% acetic acid solution (adjusted to pH 3.5 with sodium hydroxide), and 1.50 ml of 0.8% (w/v) solution of TBA, 15 µl of butylated hydroxytoluene to 0.20 ml of the brain homogenates. The final volume was made up to 4.0 ml with distilled water. The vials were tightly capped. The solution was mixed and heated in a water bath at 100°C for 60 min. The vials were next cooled with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1;v/v) were added and shaken vigorously. The material in the vials was mixed again and centrifuged at 4000 rpm for 15 minutes. After centrifugation, the organic layer was taken and its absorbance was measured against water (as blank) at 532 nm. A standard curve with different concentrations of tetraethoxypropane (TEP) (1-6 nanomoles) was prepared. Tetraethoxypropane was used as an external standard, and the occurrence of lipid peroxidation was expressed as nanomoles of malondialdehyde formed/g of fresh tissue by using TEP standard curve.

Histopathology of the brain. Discrete regions of the brain of both experimental and control rats were fixed in 10% formalin and embedded in paraffin, and were cut into 3-5 mm thick sections. The sections were stained with hematoxylin and eosin and examined for neuron cell nuclei. Myelin fibres staining was performed in Luxol Fast Blue-Cresyl Violet.[24] Demonstration of degenerated myelin was in Luxol Fast Blue-Oil Red.[25] The sections were examined by a pathologist without knowledge of the treatment.

Statistical analysis. The results were represented as mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA). When the analysis indicated a significant difference ($p<0.05$) the treated groups were compared to their respective controls. Statistical analysis were performed by F-test homogeneity of variance followed by t-test.[26]

Results. The signs of vanadium toxicity in the rats displayed ruffled hairs, pallor, lameness, difficulty in moving hind limbs, motoric disturbances, convulsions, muscular fasciculation, asphxia, severe ataxia, lethargy and diarrhoea as reported by Haider and Fakhri.[16] The rats treated with ascorbic acid alone developed skin dermatitis at the site of injection followed by diarrhea on the last day of treatment. The saline controls or ascorbic acid + vanadium-treated rats showed no evidence of observable toxic effects.

Body weight and mortality. **Table 1** shows the effect if L-ascorbic acid on the body weight of different groups of rats used in the present study. **Table 2** embodies the influence of L-ascorbic acid on the whole brain weight of the rats following vanadium intoxication. Effect of L-ascorbic acid on lipid peroxidation in rat brain after vanadium intoxication has been demonstrated in **Table 3**. The administration of L-ascorbic acid alone inhibited occurrence of lipid peroxidation in the following order: hippocampus (-35%; $p<0.001$) >medulla-pons (-13%) <mid-brain (-9%) >hypothalamus (-5%). Vanadium intoxication, however, resulted in significant increases in occurrence of lipid peroxidation in the following sequence: Mid-brain (+44%; $p<0.001$) > hippocampus (+40%; $p<0.001$) >medulla-pons (+36%; $p<0.001$) >hypothalamus (+32%; $p<0.001$) compared to saline controls. The simultaneous administration of L-ascorbic acid + vanadium revealed significant inhibition in the occurrence of lipid peroxidation compared to vanadium-intoxicated group. The sequence was: medulla-pons (-29%; $p<0.001$) >mid-brain (-28%; $p<0.001$) >hypothalamus (-23%; $p<0.001$) >hippocampus (-18%; $p<0.05$). Effect of L-ascorbic acid on the concentration of ascorbic acid in rat brain regions after vanadium intoxication has been shown in **Table 4**. L-ascorbic acid treatment resulted in significant increase in the ascorbic acid levels compared to the saline controls in the following sequence: hypothalamus (+75%; $p<0.001$) >mid-brain (+71%; 0.001) >hippocampus (+38%; p<-0.05) >medulla-pons (+34%; $p<0.05$). Furthermore, vanadium intoxicated group exhibited remarkable elevations in the ascorbic acid concentration **(Table 4)** against the saline controls in the following sequence: hypothalamus (+174%; $p<0.01$) >mid-brain (+149%; $p<0.001$) >hippocampus (+98%, $p<0.001$) >medulla-pons (+84; $p<0.001$). The concentration of ascorbic acid was depleted **(Table 4)** compared to the saline controls after concomitant (L-ascorbic acid + vanadium) treatment in the following order: medulla-pons (-54%; $p<0.001$) >hippocampus (-41%; $p<0.001$) >mid-brain (-38%; $p<0.001$) >hypothalamus (-13%). However, this group revealed further depletion in ascorbic acid concentration with respect to ascorbic acid controls in the following sequence: medulla-pons (-66%; p <.001) >mid-brain (-64%; $p<0.001$) >hippocampus (-58%; p <0.001), >hypothalamus (-50%; p < 0.001). The co-treatment (L-ascorbic acid + vandium) further exhibited decreases in the ascorbic acid levels when compared with vanadium-intoxicated group in the following sequence: mid-brain or medulla-pons (-75%; $p<0.001$) >hippocampus (-70%; $p<0.001$) > hypothalamus (-68%; $p<0.001$). **Table 5** embodies the effect of L-ascorbic acid on the activity of G6PDH in brain regions after vanadium intoxication. The vanadium toxicity significantly inhibited the activity of G6PDH compared to the saline controls. The mid-brain showed maximum inhibition (-37%;

Table 1 - Changes in body weight and incidence of mortality in rats following administration of L-ascorbic acid (1.0g/kg body weight). Vanadium (2.5 mg/kg body weight) alone or simultaneously by intraperitoneal route for 8 consecutive days. Control group was injected with normal saline 1.0 ml/kg body weight.

Group	Treatment	Initial body weight (g)	Final body weight (g)	Loss or gain of weight per day (g)	P*	Mortality
1	Normal saline (controls)	396 ± 42.57	400.67 ± 42.4	-	> 0.2	0/22
2	L-ascorbic acid	332.3 ± 90.06	377.38 ± 77.70	+5.63	< 0.001	0/22
3	Vanadium	276.5 ± 35.35	199.47 ± 66.72	-9.62	< 0.001	4/24
4	L-Ascorbic acid + vanadium	303 ± 23.33	304.3 ± 23.5	_	> 0.005	0/12

P* value as calculated between initial and final body weight. Each value is mean ± standard deviation of 12 to 24 observations

Table 2 - Effect of L-ascorbic acid on the whole brain weight after vanadium intoxication.*

Group	Treatment	Brain weight (g)
1	Normal saline (Controls)	1.77 ± 0.062
2	L-ascorbic acid	1.76 ± 0.044
3	Vanadium	1.479^{ab} ± 0.066
4	L-ascorbic acid + vanadium	1.69^{c} ± 0.048

* L-ascorbic acid (1.0/kg body weight), Vanadium (2.5 mg/kg body weight) alone or concomitantly were injected by intraperitoneal route for 8 consecutive days. Control group was injected with normal saline 1.0 ml/kg body weight
a - significantly different from controls (p<0.001)
b - significantly different from group treated with L-ascorbic acid (p<0.001)
c - significantly different from group treated with vanadium (p<0.001)
values are mean ± standard deviation of 16 observations

Table 3 - Effect of L-ascorbic acid on lipid peroxidation in different regions of rat brain after vanadium intoxication.*

Treatment/brain regions	Control	Ascorbic acid	Vanadium	Ascorbic acid + vanadium
	Lipid Peroxidation (nmol malondialdehyde formed/g of fresh tissue)			
Hippocampus	232.5 ± 28.09	150^{a} ± 19.09	325^{ab} ± 0.37	267^{abc} ± 19.9
Hypothalamus	225.62 ± 11.5	215 ± 16.29	297.5^{ab} ± 10.5	227.6^{c} ± 3.3
Mid-brain	236.25 ± 2.5	214.57 ± 37.43	340^{ab} ± 31.25	245^{bc} ± 18.8
Medulla-pons	233.75 ± 12.6	203.57 ± 73.30	317.5^{ab} ± 60.71	226.5^{c} ± 30.2

* L-ascorbic acid (1.0g/kg body weight), vanadium (2.5mg/kg body weight) alone or concomitantly were injected by intraperitoneal route for 8 consecutive days. Control group was injected with normal saline 1.0 ml/kg body weight.
a - significantly different from controls (p <0.05).
b - significantly different from group treated with ascorbic acid (p<0.05).
c - significantly different from group treated with vanadium (p<0.05)
Values are mean ± standard deviation of 16 observations

Table 4 - Effect of L-ascorbic acid on the concentration of ascorbic acid in the rat brain after vanadium administration.*

Treatment/brain regions	Control	Ascorbic acid	Vanadium	Ascorbic acid + vanadium
	Ascorbic acid (μg/g of fresh tissue)			
Hippocampus	315 ± 62.93	440^{a} ± 73.14	623^{ab} ± 32.1	184^{abc} ± 11.10
Hypothalamus	217 ± 67.55	380^{a} ± 30.59	595^{ab} ± 12.21	188^{abc} ± 8.36
Mid-Brain	220 ± 57.44	377^{a} ± 61.30	548^{ab} ± 36.1	136^{abc} ± 20.73
Medulla-Pons	228 ± 65.24	305^{a} ± 62.04	420^{ab} ± 26.5	104^{abc} ± 5.4

* L-ascorbic acid (1.0g/kg body weight), vanadium (2.5mg/kg body weight) alone or concomitantly were injected by intraperitoneal route for 8 consecutive days. Control group was injected with normal saline 1.0 ml/kg body weight.
a - significantly different from controls ($p < 0.05$).
b - significantly different from group treated with ascorbic acid ($p<0.05$).
c - significantly different from group treated with vanadium ($p<0.05$)
Values are mean ± standard deviation of 16 observations

Table 5 - Effect of L-ascorbic acid on the activity of glucose-6-phosphate dehydrogenase in the rat brain after vanadium administration.*

Treatment/brain regions	Control	Ascorbic acid	Vanadium	Ascorbic acid + vanadium
	Glucose-6-phosphate dehydrogenase (Milli Unit/g of tissue)			
Hippocampus	69.57 ± 5.18	70.2 ± 3.7	56.38^{ab} ± 7.05	60.93^{ab} ± 4.11
Hypothalamus	64.59 ± 1.78	63.4 ± 5.5	55.35^{ab} ± 8.66	59.1 ± 4.71
Mid-brain	72.61 ± 19.71	71.36 ± 15.63	45.33^{ab} ± 9.15	53.26^{abc} ± 4.11
Medulla-pons	69.3 ± 8.3	67.20 ± 3.5	47.68^{ab} ± 13.96	50^{ab} ± 0.99

* L-ascorbic acid (1.0g/kg body weight), vanadium (2.5mg/kg body weight) alone or concomitantly were injected by intraperitoneal route for 8 consecutive days. Control group was injected with normal saline 1.0 ml/kg body weight.
a - significantly different from controls ($p < 0.05$).
b - significantly different from group treated with ascorbic acid ($p<0.05$).
c - significantly different from group treated with vanadium ($p<0.05$)
Values are mean ± standard deviation of 16 observations

Table 6 - Effect of L-ascorbic acid on the activity of acetylcholinesterase in the rat brain after vanadium administration.*

Treatment/brain regions	Control	Ascorbic acid	Vanadium	Ascorbic acid + vanadium
	Acetycholinesterase (Moles /min /ml)			
Hippocampus	0.84 ± 0.14	1.8^{a} ± 0.01	0.81^{b} ± 0.03	1.03^{abc} ± 0.08
Hypothalamus	1.13 ± 0.06	2.1^{a} ± 0.03	1.07^{b} ± 0.06	1.53^{abc} ± 0.09
Mid-brain	1.18 ± 0.10	1.90^{a} ± 0.01	0.90^{ab} ± 0.01	1.34^{abc} ± 0.05
Medulla-pons	1.13 ± 0.1	1.84^{a} ± 0.03	1.03^{ab} ± 0.05	1.46^{abc} ± 0.01

* L-ascorbic acid (1.0g/kg body weight), vanadium (2.5mg/kg body weight) alone or concomitantly were injected by intraperitoneal route for 8 consecutive days. Control group was injected with normal saline 1.0 ml/kg body weight.
a - significantly different from controls ($p < 0.05$).
b - significantly different from group treated with ascorbic acid ($p<0.05$).
c - significantly different from group treated with vanadium ($p<0.05$)
Values are mean ± standard deviation of 16 observations

p<0.001) followed by the medulla-pons (-30%; p<0.001) >hippocampus (-19%; p<0.05), and the hypothalamus (-15%; p<0.05). The concomitant administration of L-ascorbic acid + vanadium also demonstrated inhibition of G6PDH activity **(Table 5)** compared to saline controls. The medulla-pons exhibited inhibition by -27% (p<0.05) followed by mid-brain (-26%; p<0.05), hippocampus (-12%; p<0.01), but hypothalamus showed inhibition by only 7%. However, L-ascorbic acid + Vanadium- treated group revealed reversal in the enzyme activity towards normal values with compared to vanadium-intoxicated group. The sequence was mid-brain (+18%; p<0.05) >hippocampus (+8%) >hypothalamus (+7%) >medulla-pons (+4%). Effect of L-ascorbic acid on activity of AChE in brain regions after vanadium intoxication has been presented in **Table 6**. The activity of acetycholinesterase enzyme in L-ascorbic acid-treated group with respect to saline controls was remarkably elevated in the hippocampus (+114%; p<0.001) followed by hypothalamus (+86%; p<0.001), medulla-pons (+63%; p<0.001), and the mid-brain (+61%; p<0.001). In the vanadium-intoxicated group, however, enzyme activity was inhibited compared to saline controls in the following sequence: mid-brain (-24%, p<0.05) >medulla-pons (-9%) >hypothalamus (-5%) >hippocampus (-4%). Co-treatment (L-ascorbic acid + vanadium) led to significant elevations in the enzyme activity when compared with saline controls in the following sequence: hypothalamus (+35%; p<0.001) >medulla-pons (+29%; p<0.001) >hippocampus (+23%; p <0.001) >mid-brain (+14%; p<0.05). Co-treatment group further demonstrated reversal of the enzyme activity towards normal levels against vanadium intoxicated rats. The maximum reversal was observed in the mid-brain (+49%; p<0.001) followed by hypothalamus (+43%; p<0.001), medulla-pons (+41%, p<0.001), and hippocampus (+27%, p <0.05).

Histopathological alterations in the rat brain after vanadium exposure. We attempted to visualize myelin structure in the hypothalamus region of the brain after administration of vanadium (2.5 mg/kg body weight x 8 days) alone or concomitantly with L-ascorbic acid (1.0 g/kg body weight x 8 days). The hypothalamus of saline controls showed large neurones having vesicular nuclei, prominent nucleoli and a coarsely granular cytoplasm **(Figure 1)**. The central white matter (myelin sheath) contained prominent nuclei and few small vacuoles **(Figure 2)**. The rats administered vanadium showed disappearance of Nissl substance around the nucleus and the central chromatolysis spreading throughout the cell. The nucleus moved away from the axonal hillock and acquired an eccentric position at the peripheral margin of the cell **(Figure 3)**. Vanadium exposure also induced neuronal edema **(Figure 4)**. The edematous neuron had a distended, watery, and lacy cytoplasm with eccentric nuclei. The swelling of the white matter was the result of edematous change of the myelin sheath in white matter after vanadium intoxication **(Figure 5)**. Simultaneous treatment of rats with L-ascorbic acid + vanadium also demonstrated marked vacuolation of white matter and edematous neuron.

Discussion. In the present investigation, we injected 2.5 mg elemental vanadium/kg body weight for 8 consecutive days by i.p. route. Earlier,[10] also we injected the same dose of vanadium to the rats for 7 consecutive days. This dose-induced neurological signs, such as muscular fasciculations, asphysia, lethargy and ataxia, and lameness, difficulty in moving hind limbs, motoric disturbances followed by diarrhea. We attempted to investigate the antioxidant effect of ascorbic acid (Vitamin C) against vanadium neurotoxicity in this study. We administered 1.0 g L-ascorbic acid/kg body weight to the rats alone or concomitantly with vanadium for 8 consecutive days. Eichbaum et al[18] injected similar dose to the rats to protect against strychnine poisoning and in tetanus as well. Vanadium administration resulted in loss of body and brain weights. The body weight loss and akinesia in our study could be attributed to refusal to take food by animals and deficit of energy (calories). At times of dietary restriction there is rapid loss of tissue protein and adaptive changes take place to conserve protein. It is likely that body weight loss was also associated largely with dehydration through diarrhea. Vanadium intoxication evoked neuronal cell loss, as shown in **Figures 3 & 4** of histopathology, consequently, the brain weight was reduced. In this investigation, co-treatment (L-ascorbic acid + vanadium) demonstrated protective effects against vanadium toxicity as all signs and symptoms, produced by vanadium, were reversed.

A previous study[16] from this laboratory has revealed that the administration of vanadium (2.5, 3.5 or 5.0 mg/kg) by i.p. route for 7, 3 or 2 consecutive days resulted in significant enhancement of lipid peroxidation in the discrete brain areas. The results of our study also demonstrated that exposure to vanadium (2.5 mg/kg body weight), i.p., for 8 consecutive days resulted in significant increase in the occurrence of lipid peroxidation in the discrete areas of rat brain, when compared with saline controls or L-ascorbic acid treated group. The data is in good agreement with our[16] aforementioned report. **Table 3** of this study demonstrated that administration of L-ascorbic acid alone or concomitantly with vanadium inhibited endogenous lipid peroxidation when compared with saline controls or vanadium-intoxicated group. The results of this study are in congruence with our previous report,[16] where α-tocopherol (vitamin E) protected the rat brain against vanadium-initiated lipoperoxidative

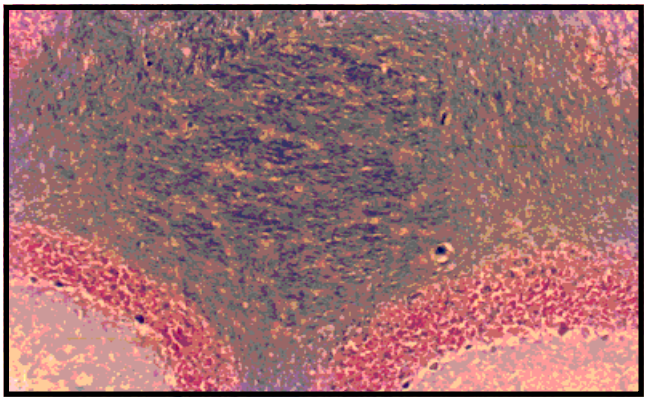

Figure 1 - The myelin sheath in white matter of rat hypothalamus, 8 days after normal saline administration, showing large neurons with vascular nuclei. Hematoxylin and Eosin. Original magnification x 100.

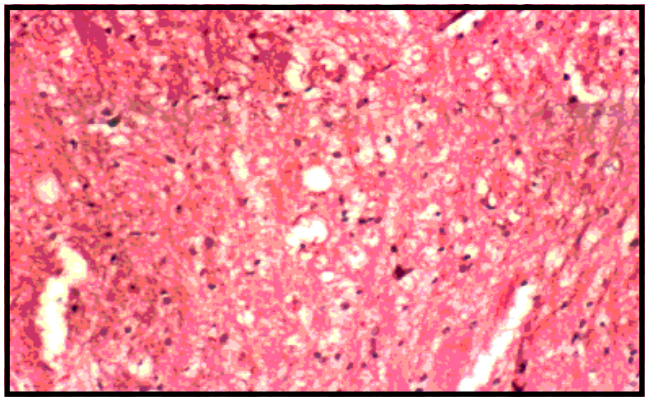

Figure 4 - The swelling and very marked edematous neuron had distended, watery, and lacy cytoplasm with eccentric nuclei in rat hypothalamus white matter (myelin sheath), 8 days after vanadium (2.5 mg/kg body weight) administration. Hematoxylin and Eosin. Original magnification x 200.

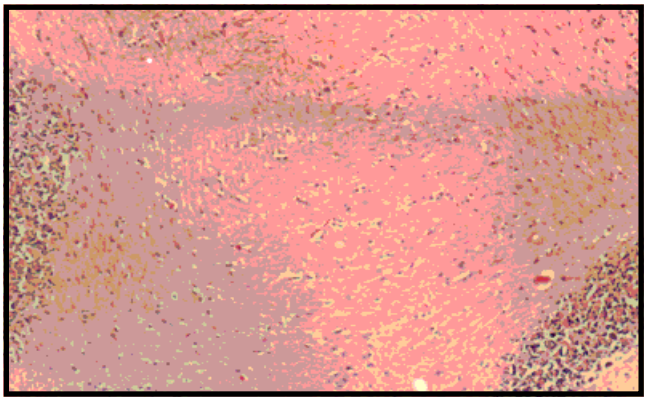

Figure 2 - The myelin sheath in the white matter of rat hypothalamus, 8 days after normal saline administration, showing neurons with prominent nuclei and few small vacuoles. Hematoxylin and Eosin. Original magnification x 200.

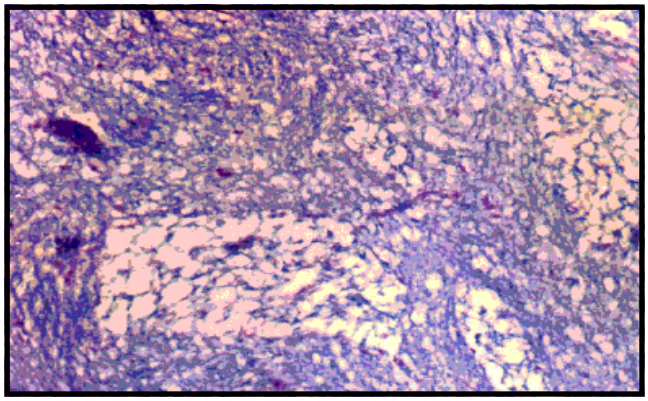

Figure 5 - The swelling and vaculation of white matter-induced by vanadium (2.5 mg/kg body weight) x 8 days intoxication in the rat hypothalamus. The swelling of white matter was the result of intramyelin edema. Luxol Fast Blue. Original magnification x 100.

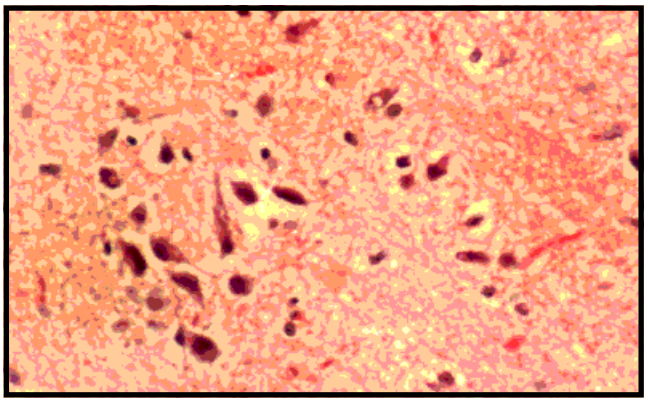

Figure 3 - Central chromatolysis. Neurons in the rat hypothalamus showing displacement of the nucleus to the periphery of the cell, 8 days after vanadium (2.5 mg/kg body weight) administration. There is dispersion of Nissl substance in the neuronal cytoplasm. Hematoxylin and Eosin. Original magnification x 400.

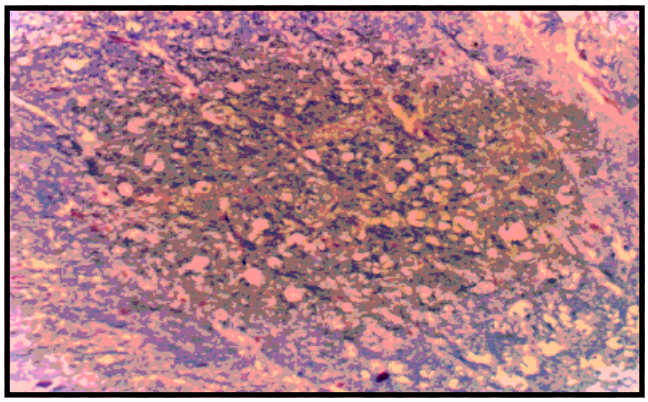

Figure 6 - The marked vacuolation of the white matter and of neurons following co-treatment with L-ascorbic acid (1.0 gm/kg body weight) + vanadium (2.5 mg/kg body weight) x 8 days. Luxol Fast Blue. Original magnification x 100.

damage. The mechanism involved may be explained on the basis of the fact that Vitamin E protects cell membrane from peroxidative damage via conversion in to Vitamin E radical. Ascorbate can react with Vitamin E radical and regenerate Vitamin E in its original chemically active from.[27]

Our investigation has shown that the activity of G6PDH was significantly inhibited in discrete rat brain regions following vanadium intoxication. This phenomenon might have perturbed route of glucose oxidation, and production of NADPH, which is involved in lipid synthesis. The rats treated concomitantly (L-ascorbic acid + vanadium) exhibited reversal in the activity of G6PDH towards their normal values in comparison to vanadium-intoxicated group. It is thus likely that co-treatment might have raised the availability of NADPH via hexosemonophosphate shunt in the brain. The inhibition of lipid peroxide in this co-treated group suggested that the main effect of L-ascorbic acid was an accelerated removal of lipid peroxides, accumulated as a result of vanadium metal toxicity.

It is well documented that AChE activity is influenced by changes in membrane microenvironment.[28] Previously, it has been reported that cholinesterase activity in the rat brain was reduced by the i.p. administration of 1-10 mg vanadyl sulfate/kg body weight.[29] In our study also the activity of AChE enzyme was inhibited in the discrete regions of the rat brain following vanadium exposure. It is thus likely that membrane fluidity was altered as a consequence of lipid peroxidative damage. The reversal of the diminished levels of AChE towards their normal values in the L-ascorbic acid + vanadium administered group signified the efforts of ascorbic acid for a possible repair of the damaged membrane, its fluidity and integrity. The vanadium intoxicated group in our study exhibited remarkable elevations in the ascorbic acid concentration compared to the L-ascorbic acid-treated controls. This event is probably associated with amelioration of vanadium-induced toxicity.[11] Secondly, the adaptive responses to the raised content of peroxidized lipids after vanadium exposure implicated increased absorption of ascorbic acid via cerebrospinal fluid. There are evidences that concentration of this vitamin is altered in varying degrees under the influence of the metals.[30] The noteworthy findings of L-ascorbic acid+vanadium treatment were reversal of brain ascorbic acid concentration to normal values compared to saline controls or vanadium intoxicated groups. It is, therefore, speculative that the depletion of ascorbic acid content in the brain could be a reason for its greater utilisation in reducing the vanadium toxicity. The neurohistopathology performed in the hypothalamus region of the rat brain had defined the nature and characteristics of the damage produced by vanadium toxicity compared to the normal saline controls. The central chromatolysis **(Figures 3 & 4)**, the neuronal cell body swelling with a dissolution of a Nissl substance around the nucleus showed axonal injury after vanadium toxicity. These findings implicated cell degeneration and death without recovery. Our investigation also revealed that vanadium neurotoxicity produced edematous changes and marked vacuolation of the central white matter (myelin) in the hypothalamus region of rat brain compared to normal saline controls **(Figure 5)**. Co-treatment (L-ascorbic acid + vanadium), did not reveal any significant effect on the vanadium-induced axonal injury **(Figure 6)**. Our results suggested that vanadium produced its deleterious effects by lipoperoxidative damage and interfering with enzyme activities, responsible for maintaining integrity of neuronal cell membrane, in the discrete regions of the rat brain. Although ascorbic acid reduced the toxicity of vanadium, much remains to be learned regarding the beneficial effects of this vitamin during the course of our future research work.

Acknowledgment. The authors are thankful to Prof. R. N. Visweswara, Department of Pathology, for critical review of histopathological slides.

References

1. Banhegyi G, Braun L, Csala M, Puskas F, Mandl J. Ascorbate metabolism and its regulation in animals. *Free Radic Biol Med* 1997; 23: 793-803.
2. Fox MRS. Protective effects of ascorbic acid against toxicity of heavy metals. *Ann N Y Acad Sci* 1975; 258: 144-150.
3. Chakraborty D, Bhattacharyya A, Mujumdar K, Chatterjee K, Chatterjee S, Chatterjee A et al. Studies on ascorbic acid metabolism in rats under chronic toxicity due to organophosphorus insecticides: Effects of supplementation of ascorbic acid in high doses. *J Nutr* 1978; 108: 973-980.
4. Chatterjee GC, Rudra Pal D. Metabolism of L-ascorbic acid in rats under in vitro administration of mercury: Effect of L-ascorbic acid supplementation. *International Journal of Vitamin and Nutritional Research* 1975; 45: 284-292.
5. Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Ames MM. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-bind comparison. *N Engl J Med* 1985; 312: 137-141.
6. Block G. Vitamin C and cancer prevention: The epidemiological evidence. *Am J Clin Nutr* 1991; 53: 2705-2825.
7. Cook JD, Monsen ER. Vitamin C, the common cold and iron absorption. *Am J Clin Nutr* 1977; 30: 235-241.
8. Levine M. New concepts in the biology and biochemistry of ascorbic acid. *N Engl J Med* 1986; 314: 892-902.
9. Harakeh S, Jariwalla RJ, Pauling L. Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. *Proc Natl Acad Sci* 1990; 87: 7245-7249.
10. Spectro R, Lorenzo AV. Specificity of ascorbic acid transport system of the central nervous system. *Am J Physiol*

1974; 225: 1468-1472.
11. Bonorden WR, Pariza MW. Antioxidant nutrients and protection from free radicals. In: Kotsonis FN, Mackey M, Helle J, editors. Nutritional Toxicology. New York (NY): Raven Press; 1994. p. 19-33.
12. Bycakowski JZ, Kulkarni AP. Vanadium redox cycling, lipid peroxidation and co-oxygenation of benzo (9) pyrene-7,8-dihydrodiol. *Biochim Biophys Acta* 1992; 1125: 134-141.
13. Halliwall B. Reactive oxygen species and the central nervous system. *J Neurochem* 1992; 59: 1609-1623.
14. Berman E. Toxic Metals and their Analysis. In: Berman E, editor. Philadelphia (PA): Hayden; 1980.
15. Sasi MM, Haider SS, El-Fakhri M, Ghwarsha KM. Microchromatographic analysis of lipids, protein and occurrence of lipid peroxidation in various brain areas of vanadium exposed rats: A possible mechanism of vanadium neurotoxicity. *Neurotoxicology* 1994; 15: 413-420.
16. Haider SS, El-Fakhri M. Action of alpha tocopherol on vanadium-stimulated lipid peroxidation in rat brain. *Neurotoxicology* 1991; 12: 79-89.
17. Domingo JL, Llobet JM, Thomas JM, Corbella J. Influence of chelating agents on the toxicity, distribution and excretion of vanadium in mice. *J Appl Toxicol* 1986; 6: 377-391.
18. Eichbaum FW, Guedes AO, Neto JP, Carvalho FV. Protecting effect of ascorbic acid in strychnine poisoning and in tetnus (Experiments in fish, mice and rats). In: Hank A, Ritzel G, editors. Re-evaluation of Vitamin C. Verlag (DE): Hans Huber; 1977. p. 31-40.
19. Glowinski J, Iverson IL. Regional studies on catecholamines in the rat brain. I. The disposition of (3H) norepinephrine, dopamine and (3H) Dopa in various regions of the rat brain. *J Neurochem* 1966; 13: 665-669.
20. Lohr GW, Walter HD. Glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. Vol III. 2nd ed. New York (NY): Academic Press; 1974. p. 636.
21. Plummer DT. The assay of acetylcholinesterase. In: Plummer DT, editor. An Introduction to Practical Biochemistry. London (UK): McGraw Hill; 1987. p. 87-88.
22. Kyaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. *Clin Chim Acta* 1978; 86: 153-157.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 45: 351-358.
24. Kluvery H, Barrera E. A method for a combined staining of cells and fibers of the nervous system. *J Neuropathol Exp Neurol* 1953; 12: 400-403.
25. Swank RL, Davenport HA. Chlorate-osmic formation method for staining degenerate myelin. *Stain Technol* 1935; 10: 87-90.
26. Kirkwood BR. Essentials of Medical Statistics. In: Kirkwood BR, editor. Oxford (UK): Blackwell Scientific Publications; 1983. p. 1-10.
27. Mayes PA. Structure and function of the water soluble vitamins. In: Murry RK, Granner DK, Mayes PA, Rodwell VW, editors. Harper's Biochemistry. New Jersey (NJ): Prentice Hall International Inc; 1993. p. 586-587.
28. Ott P. Membrane acetylcholinesterase: Purification, molecular properties and interactions with amphilic environment. *Biochem Biophys Acta* 1985; 822: 357-392.
29. World Health Organization (WHO) Technical Report Series. Vanadium. Environmental Health Criteria. No. 81: Geneva; 1988.
30. Hughes RE. Vitamin C recent aspects of its physiological and technological importance. In: Birch CG, Parker K, editors. London (UK): Applied Science Publishers Ltd; 1974. p. 68-74.