

Protective Role of Vitamin C against Neurolathyrism in Guinea pigs

Pages with reference to book, From 2 To 5

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Abstract

Guinea-pigs maintained on a vitamin C deficient diet for 30 days lost body weight (13.3%) and had reduced level of vitamin C (48.9%) in the serum. These guinea-pigs demonstrated a consistent pattern of neurological signs of neurolathyrism when administered intraperitoneally with an extract of *Lathyrus sativus* seeds, equivalent to 31.5 rmg of active toxin, B - N-oxalyl- L-a, B-diaminopropionic acid (ODAP). On the other hand, animals in the control group which received the vitamin (15 mg/day/head) throughout the experimental period were resistant to these symptoms after the same level of the toxin administration. The results therefore demonstrate that vitamin C has a protective role against neurolathyrism in adult guinea-pigs (JPMA 35:2. 1985).

Introduction

Neurolathyrism is a nervous disease of the humans caused by excessive consumption of *Lathyrus sativus* (Khesari dhal) (200 g or more per day) for prolonged periods¹⁻⁴ During drought when most other crops fail, the poor eat *L. sativus* seeds rather than starve. Although epidemiological studies clearly link onset of paralysis to prolonged excessive consumption of *L. sativus* seeds, the failure of the toxin, B-Noxalyl-L-a, B-diaminopropionic acid (ODAP), present in *L. sativus* seeds to affect adult experimental animals has raised questions regarding the role of this neurotoxin in the etiology of neurolathyrism⁴.

Little information is available on the dietary deficiency in animals prone to neurolathyrism. Dietary surveys¹ carried out in the epidemic areas have shown that there is no evidence of any dietary protein or calorie deficiency although the diets of the neurolathyrism victims usually lack in vitamin C and A, and fat¹. Diets of lathyrism patients were deficient in vitamin C⁵ which was thought to be playing some role in the causation of lathyrism. It was therefore considered worthwhile to study the effects of dietary vitamin C depletion and supplementation in producing the symptoms of neurolathyrism in an animal susceptible to this disease e.g. the guinea-pig.

We undertook this experiment on the acute neurotoxicity of *L. Sativus* seeds in the guinea pig after observing that human subjects afflicted with the disease consumed a nutritionally inadequate diet specially in vitamin C⁶. A population well-nourished in respect of the vitamin C would not develop neurolathyrism in spite of the use of Khesari as a principal item in the diet.

Material and Methods

Animals: Twenty healthy adult white guinea-pigs of both sexes (weighing between 240 and 350 g) and of various ages collected from the International Center for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B), were used for the study. The animals were divided into two groups: one received a diet supplemented with vitamin C (15 mg/day! head for 30 days) and the other received a diet without vitamin C. Both groups were fed on boiled acer arietinum (desi chana) seeds and a vitamin-salt mixture⁷ and housed two per cage under proper hygienic conditions. The diets and water were given ad

libijum. Body weights of the animals were recorded every 6 days. Serum vitamin C levels⁸ were also measured at these intervals.

Preparation of seed extract: *L. sativus* seed toxin was extracted by steeping the seed powder (200 g) in 800 ml 75% ethanol and then dissolved in 10 ml distilled water after evaporating the alcohol present in the filtrates⁹. The content of the toxin in the seed extract was determined by a specific sensitive colour reaction¹⁰

Toxin administration The animals in each group were divided into two subgroups on the 30th day from the beginning of the experiment. The subgroups then were intraperitoneally injected with either 3ml of normal saline or 3 ml of *L. sativus* seed extract (equivalent to 31.5 mg toxin) freshly prepared as above. The animals were observed for any symptoms of neuropathism or other clinical changes following the injection.

Results and Discussion

Guinea-pigs maintained on a diet supplemented with vitamin C showed an increase (30%) in body weight during the 30 days of feeding, whereas those given no vitamin C registered a significant loss of body weight (13.31%) during the same period (Fig 1).

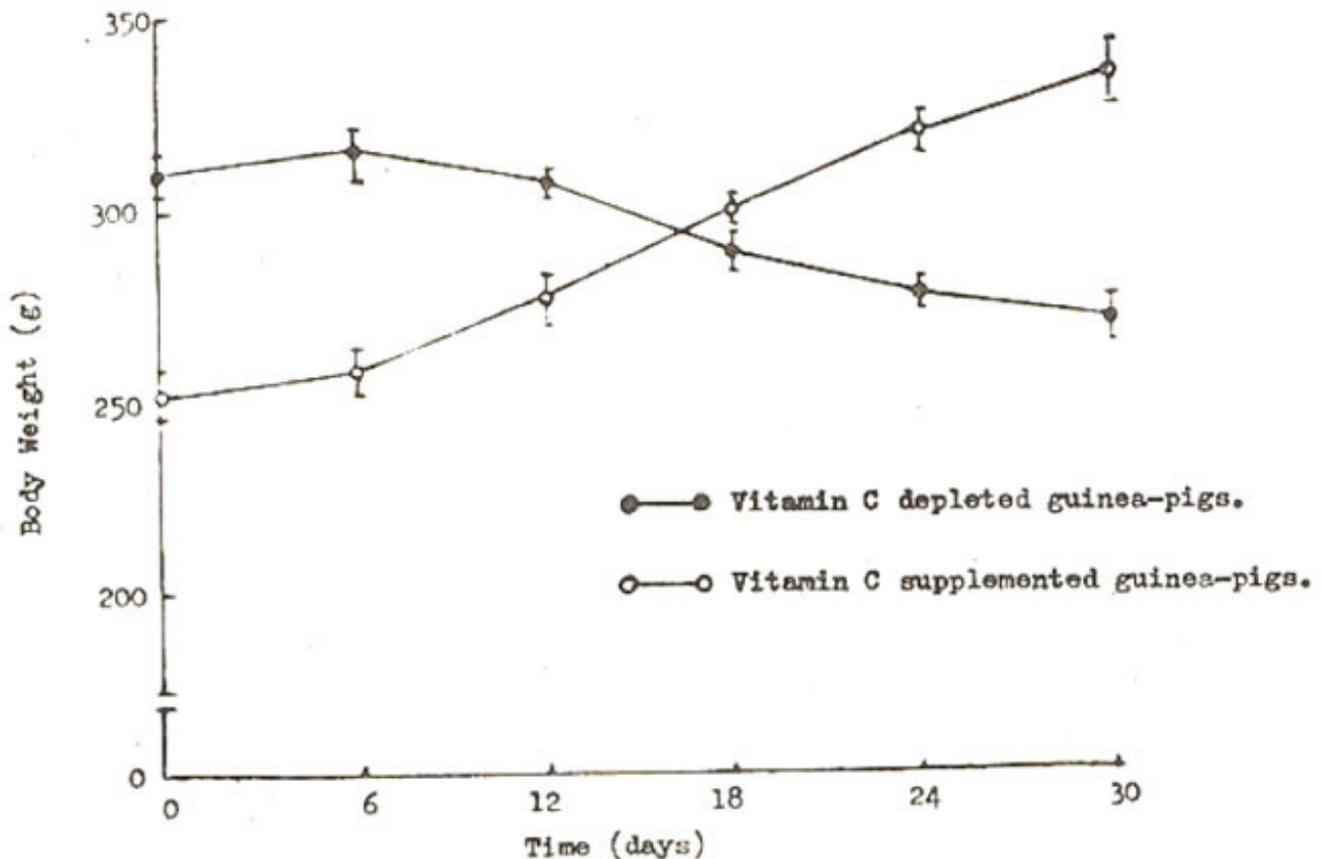


Fig. 1. Changes of body weight of guinea pigs maintained on a diet supplemented with or depleted of vitamin C. Each value is the mean of 10 animals and the bars show the standard deviation.

The mean serum vitamin C level in the vitamin depleted animals (0.23 mg/100 ml) was also significantly lower ($P < 0.001$) than that in the supplemented group (0.59 mg/100ml) after 30 days (Fig 2).

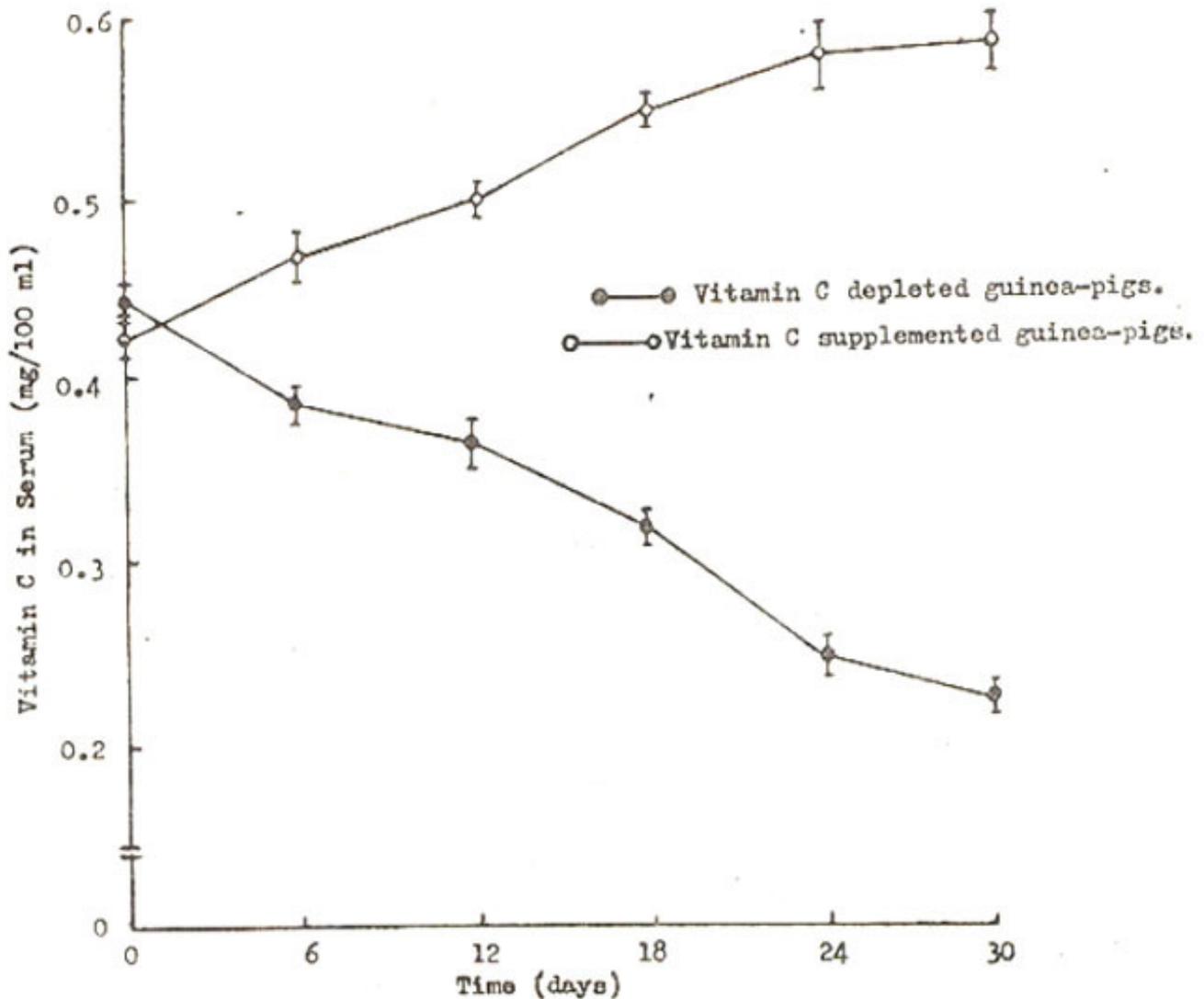


Fig.2. Serum vitamin C levels of guinea pigs maintained on a diet supplemented with or depleted of vitamin C. Each value is the mean of 10 animals and the bars show the standard deviation.

The present results clearly demonstrate that dietary depletion of vitamin C in adult guinea-pigs results in decline in serum level of the vitamin, concomitant with failure in maintenance of body weight. Clinical observations showed that the crude alcoholic extract of *L. sativus* seeds produced all symptoms related to neuropathy in the guinea-pigs given a diet without vitamin C, whereas those given the vitamin supplement were not affected (Table).

Table

Protective Action of Vitamin C Against Neurolathyrism in Adult Guinea-Pigs¹

Group		Number of animals	Diet	Observation
A-1	(Injected with saline)	5	Boiled deshi chana supplemented with all B vitamins, vit. A and D, and 15 mg/day/head vitamin C.	Normal in all respect, none showed neurological symptoms.
A-2	(Injected with saline)	5	As in A-1.	As above.
B-1	(Injected with saline)	5	As in A-1 except that vitamin C was omitted.	As above.
B-2	(Injected with toxin)	5	As in B-1.	All animals developed neurological symptoms of lathyrism within 3 hours of toxin injection.

At the end of 30-day feeding of the respective diets, the animals were injected intraperitoneally with either saline or toxin. See Materials and Methods for details of toxin preparation and administration.

The affected animals became dull and showed convulsions, tremors and ataxic gait. Paralysis of the legs developed within 3 hours after the toxin administration.

Incidence of neurolathyrism as a clinical disorder after intraperitoneal injection of *L. sativus* seed extract in the adult guinea-pigs has not been previously reported. The present experiment thus demonstrates that *L. sativus* seed extract is toxic to adult guinea-pigs and produced neural symptoms comparable to those seen in human cases of lathyrism, when serum vitamin C deficiency is prominent. Animals maintained on a vitamin C supplemented diet and thus with normal serum vitamin C level were resistant to the development of such symptoms. Not a single animal of group A (Table) which received vitamin C showed any neuropathological symptoms, while all animals in group B (which did not have vitamin C) progressively developed symptoms, while all animals in group B (which did not have vitamin C) progressively developed the symptoms of neurolathyrism within 3 hours after the extract administration.

The idea that primates (including man) which are incapable of synthesizing vitamin C and therefore depend on dietary sources of the vitamin might have metabolic differences which make them particularly vulnerable to ODAP intoxication has been implied by the extensive use of monkeys in recent work on the lathyrus toxin¹⁰⁻¹⁴ The present study shows that the guinea-pigs are similar to the monkey in susceptibility to the toxin. Because the guinea-pigs are less expensive and easy to breed in the laboratory, its use permits studies such as those described here or other biochemical studies, which would be more inconvenient or expensive with monkey.

The mechanism of vitamin C protection against neurolathyrism is not clear at present. It has previously been observed that ODAP produces toxic effects in young animals e.g. one-day old chick^{9,15} or squirrel monkey¹⁵ and not in the adult. It was also observed that neurolathyrism can be developed in adult primates when the neurotoxin is injected intrathecally i.e. directly into the central nervous system^{10,14,17,18} These observation led to the suggestion that ODAP intoxication is related to the blood-brain barrier (EBB) and not the result of unique species sensitivity. However, the results of the present experiments do not agree with the above view, because in adult guinea-pigs the BBB is considered to be mature¹⁹ BBB is in fact known to be mature in this species prenatally). The results, on the other hand, seem to be more related to the vitamin C status of the animal. It is possible that vitamin C helps in the quick detoxification of ODAP, by increasing the rate of its hydroxylation. It is also possible that vitamin C directly or indirectly prevents the toxin from binding with its specific receptor

sites in the post-synaptic membrane. Studies with labelled ODAP could clarify these possibilities. It is also necessary that detailed biochemical studies be carried out, before a comprehensive picture of the neurotoxin effect and its prevention by vitamin C is obtained.

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