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Vitamin C in the Prevention and Treatment of Frostbite

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ABSTRACT

Two series of experiments were conducted on rats to evaluate the effect of Vit C for prevention and treatment of frostbite. For studies on prevention, 45 rats were divided into 3 groups, as control, Vit C–short term and Vit C–long term. Frostbite was produced experimentally in both the hind limbs by exposing the animals at –15 °C for 1 h and the assessment of cold injury was done after 15 days. In another study on prevention, frostbite was produced on 20 rats twice, in each limb separately with and without Vit C therapy. The result showed that administration of Vit C for a short period prior to cold exposure was of no significant benefit. However, administration of high dose of Vit C for prolonged period maintained higher rectal temperature and significantly reduced the incidence of frostbite.

For studies on treatment, frostbite was produced experimentally in 4 groups of rats. Group I was treated as control, group II was administered 5 mg of Vit C/100 g bw (orally) daily for 15 days. Rapid rewarming at 37 °C on the exposed limbs of group III animals was carried out immediately after cold exposure. Combination of rapid rewarming followed by Vit C therapy was given to group IV. The degree of injury of various groups were compared statistically. Prolonged Vit C therapy as well as waterbath rewarming at body temperature immediately after cold exposure showed significant reduction in tissue damage. High dose of Vit C therapy preceded by rapid rewarming showed additional benefit in reducing the tissue loss.

INTRODUCTION

Prolonged exposure to severe cold often results in peripheral cold injuries. In civilian life it is not very common except for occasional cases seen among mountaineers, but is a major health hazard and a serious medical problem for our Armed Forces who have to operate under severe cold conditions in the snowbound areas of high altitude.

There are different forms of cold injuries, viz chillblains, trench foot, shelter foot, frostbite, etc. Out of these, frostbite is the severest form and is responsible for the loss of large number of fingers and toes of our soldiers posted to snowbound areas at high altitude. Frostbite generally occurs at freezing temperatures and below. As peripheral tissues cool faster than other parts of the body, the injury is mostly on the hands and feet[1-3] The severity of atmospheric cold and prolonged exposure to such conditions (duration) are the main factors for the occurrence of frostbite. Wind movement hasten tissue cooling.

Inspite of many research studies conducted for its prevention and treatment, frostbite continues to cause serious concern[4-9] especially for those who are posted to higher altitudes. Drug therapy as a measure of treatment is not very effective and is often cumbersome in the field areas. Therefore, additional doses of food ingredients like vitamins assume considerable importance in the prevention and treatment of frostbite for which the present study is undertaken.

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2. MATERIALS AND METHODS

One hundred forty-five albino rats of either sex with body weight ranging from 170 to 220 g were used for this study. Two series of experiments, one for prevention and the other for treatment were carried out during winter months at Delhi.

2.1 Series I

In this series, two sets of experiments were conducted. For set 1, 45 rats were divided into 3 groups of 15 each by random selection. Group A was treated as control. Group B (Vitamin (Vit) C–short term) and C (Vit C–long term) animals were administered orally with 5 mg of Vit C/100 g body weight (bw) daily for a period of 7 and 21 days respectively, prior to cold exposure. 500 mg tablet of Vit C from Glaxo Company was dissolved in 50 ml of distilled water and was administered orally with the help of a syringe as per the doses, calculated according to the body weight. Fresh solution was prepared and used each day. Frostbite was produced experimentally in both the hind limbs of all the animals using harness technique[10]. The rat was enclosed in a perspex harness suitably fitting to the body while both the hind limbs were protruding out of it and were fully exposed. The tail of the animal was also protected in a block of thermocole insulation. The rat with this set-up was exposed to severe cold at -15 ± 1 °C in a thermostatically-controlled deep-freezer for a period of 60 minutes making provision for respiratory gas exchanges. The core, paw and the ambient temperatures were recorded before starting the experiment, and every 5 minute interval thereafter, using copper-constantan thermocouples in conjunction with a Doran thermocouple potentiometer and a multiflex spot galvanometer. At the end of 1 hr, the rat was removed from the freezer and apparent changes of the paws, if any, were noted. After 15 days, the final assessment of the nature and severity of the injury was made[10], the details of which are given in Fig 1. The changes in the rectal temperature and degree of injury were then compared statistically using *t*-test and Man-Witney *U*-test.

After observing the results of this set of experiments, another group of 20 rats was selected for the next set. In this, the same animal was used as its own control and was exposed twice to cold. During the first exposure, one of the hind limbs (control limb), kept protruding outside the harness, was exposed to severe cold (-15 ± 1 °C) for producing frostbite; while the other hind limb (experimental limb) was protected inside the harness. After 15 days the severity of cold injury was assessed and classified as in the previous case. From sixteenth day onwards, the same rat was administered orally with Vit C (5 mg/100 g bw) daily for next 21 days, after which frostbite was produced on the other hind limb (experimental limb) also. Measurements of temperature and the assessment of the degree of injury was made as done earlier. Statistical comparison was made for the changes in the rectal temperature and degree of injury between the two exposures by using *t*-test and Wilcoxon matched-pairs signed-ranks test.

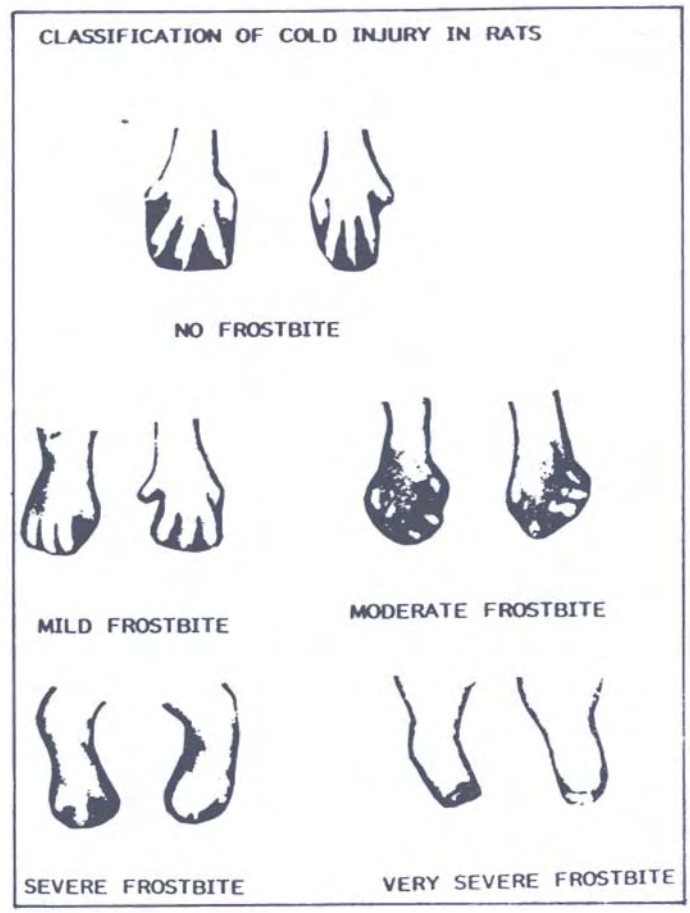

Figure 1 **Diagram showing the assessment of various degrees of cold injury (frostbite).**

2.2 Series II

In this series, 4 groups (20 each) of rats were used to see the beneficial effect of Vit C, if any, with and

without rewarming, as an immediate treatment to cold injury. Frostbite was produced experimentally in both the hind limbs of all the rats by exposing them to −15±1 °C for 1 hr as described in Series I. The first group of animals served as control and no treatment of any sort was given to them after cold exposure. After 15 days, final assessment of the degree of cold injury was made as per the criteria given earlier. Following cold exposure, the second group was treated with Vit C (orally, 5 mg/100 g bw) daily for the next 15 days and assessment for the degree of injury was made after that. In the third group, immediately after removal of the rat from the freezer both the exposed limbs were rewarmed in a constantly stirred waterbath maintained at 37 °C for a period of 30 minutes. Assessment for the extent of injury was made after 15 days. Both the hind limbs of fourth group of animals were also rewarmed at 37 °C water-bath as before and thereafter 5 mg/100 g bw of Vit C was given daily for the next 15 days. After the final assessment, the degree of cold injury of the various groups were compared statistically using Man-Witney *U*-test.

3. RESULTS

3.1 Series I

The mean initial rectal temperatures in all the three groups of the first set of experiment were identical and there was no significant difference between them statistically; the mean ± SD (standard deviation) values being 37.93 ± 0.17, 37.92 ± 0.28 and 38.01 ± 0.18 °C respectively for groups A, B and C. During the cold exposure, rectal temperature showed a significant fall in all the animals and the magnitude of fall for groups A, B and C respectively was 19.28 ± 0.81, 18.91 ± 0.73 and 17.78 ± 0.72 °C. This fall in rectal temperature was significantly ($P < 0.001$) lower for group C (Vit C–long term) as compared to the other two groups. The values of rectal temperature for group B showed slightly less fall as compared to group A, but the temperature fall was not significant (Fig. 2).

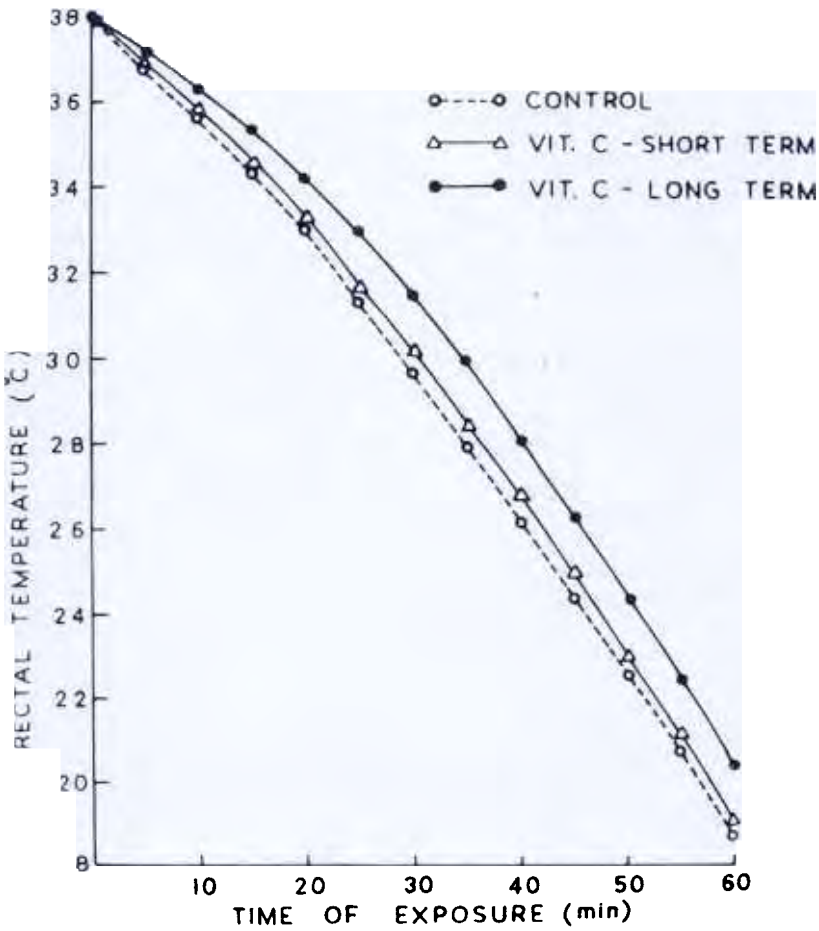

Figure 2. Pattern of fall in rectal temperature during cold exposure in control and groups treated with Vitamin C

It is seen from the results that the levels of the degree of injury in groups A and B were more or less similar and there existed no significant difference. The statistical analysis showed that the administration of Vit C for a short period of 7 days prior to cold exposure was of no benefit. However, when high dose of Vit C was administered for a prolonged period of 21 days before cold exposure, it showed a significant ($P < 0.02$) improvement in preventing the tissue damage. In the group C out of 30 paws, 10 paws (33.3 per cent) escaped cold injury and the same percentage suffered only with primary type of injury, whereas the injured were 10 and 20 per cent for group A and 13.3 and 23.3 per cent of the paws for group B respectively. In the case of groups A and B, 33.3 per cent paws suffered from severe to very severe type of injury, while in group C, only 13.3 per cent fell in these categories (Fig. 3).

The results of the second set of experiments of this series, where each animal was used as its own control, supports the observation of the previous set. It is evident from the results of this set of experiments (Fig 4) also that the administration of Vit C for a prolonged period of 21 days has shown significant ($P < 0.01$) improvement in preventing the tissue damage. The magnitude of fall in rectal temperature during first and second cold exposures was identical to that of groups A and C of the first set and was highly significant ($P < 0.001$). The fall in the rectal temperature during the first cold exposure, before the treatment was 19.17 ± 0.79 °C whereas it was 17.86 ± 0.62 °C when the animals were exposed to severe cold after 21 days of treatment with high dose of Vit C. Similarly, out of 20 experimental paws, 30 per cent (6 paws) remained free from any type of injury and in 40 per cent of paws the damage was of primary nature only whereas in control paws the corresponding percentage were 10 and 15 respectively. The paws suffered from severe and very severe degree of injury were respectively, 20 and 15 per cent for control limbs compared to 10 and 5 per cent for experimental limbs.

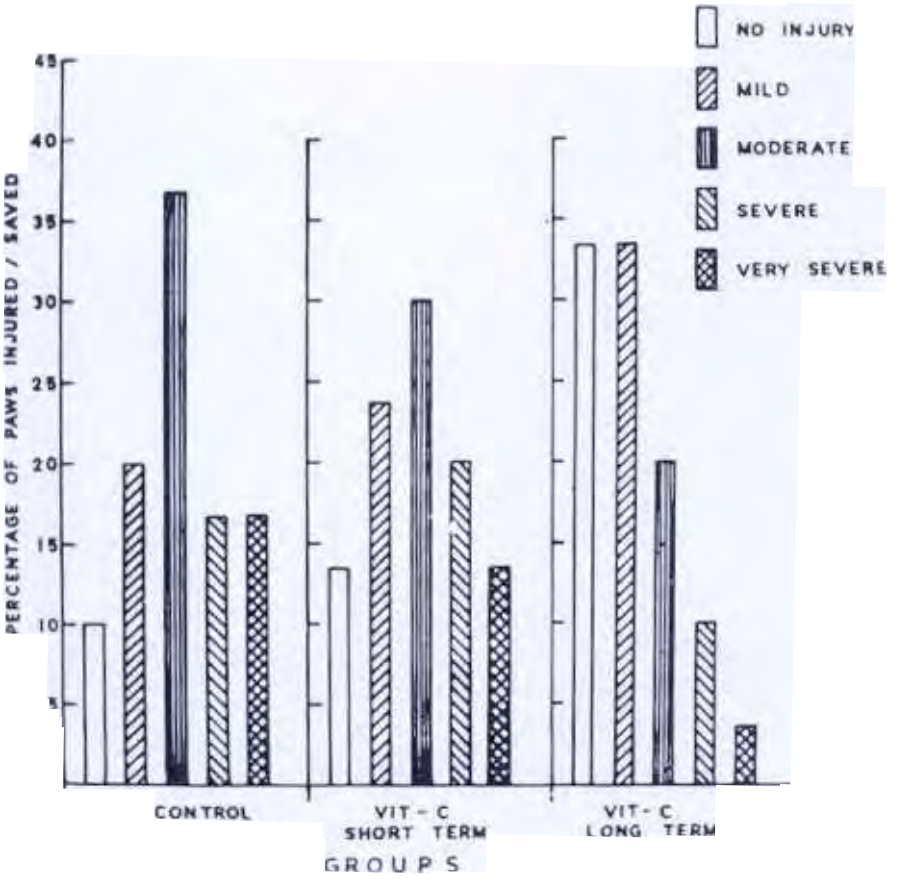

Figure 3 Percentage distribution of the degree of cold injury in control and groups treated with Vitamin C

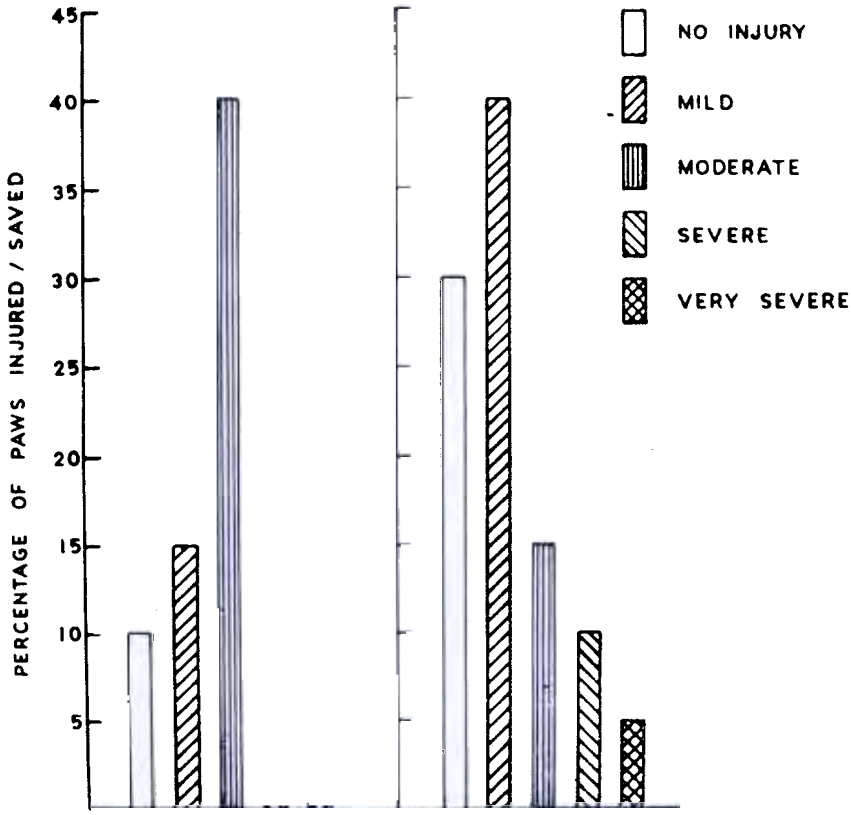

Figure 4. Percentage distribution of the degree of cold injury in control and experimental limb.

3.2 Series II

The final assessment of the nature and severity of cold injury for all the four groups of animals of this series are depicted in Fig 5. Statistical analysis showed significant ($P < 0.02$) improvement in reducing the tissue damage in Vit C treated group as compared to control. Similar level of improvement was also observed in the group where exposed limbs were rewarmed immediately after cold exposure. However, when Vit C treated group and rewarmed group were compared between themselves, there was no significant difference in the injury level, statistically. The beneficial effect of either the administration of Vit C or immediate rewarming treatment at 37 °C was apparent only when the injury level was comparatively mild or moderate in nature. But the beneficial effect of Vit C administration for 15 days preceded by rewarming at 37 °C immediately after cold exposure showed additional improvement. Out of 40 paws of this combination group, only 15 per cent (6 paws) suffered from severe to very severe type of injuries, whereas the percentage of paws affected with identical severity were 32.5, 30 and 27.5 per cent respectively for control, Vit C treated and rewarmed groups. The combination group showed a highly significant ($P < 0.002$) improvement in reducing the tissue damage as a result of rapid rewarming followed by large dose of Vit C administration as compared to control.

4. DISCUSSION

The immediate response of exposure to severe cold is peripheral vasoconstriction, mainly of small arteries and arterioles[11] leading to reduction in blood flow especially to the extremities. This makes these areas more vulnerable to cold injury, which is believed to occur partly due to anoxia resulting from circulatory insufficiency and partly due to direct freezing effect of cold on the exposed parts[1-3,12]. Insufficient circulation leads to statis in the capillaries and this blocks these small vessels which results in thrombosis. Thrombosis aggravates anoxia and leads to necrosis[1,13,14]. At this stage a slight increase in the extremity bloodflow may prevent complete anoxia and necrosis.

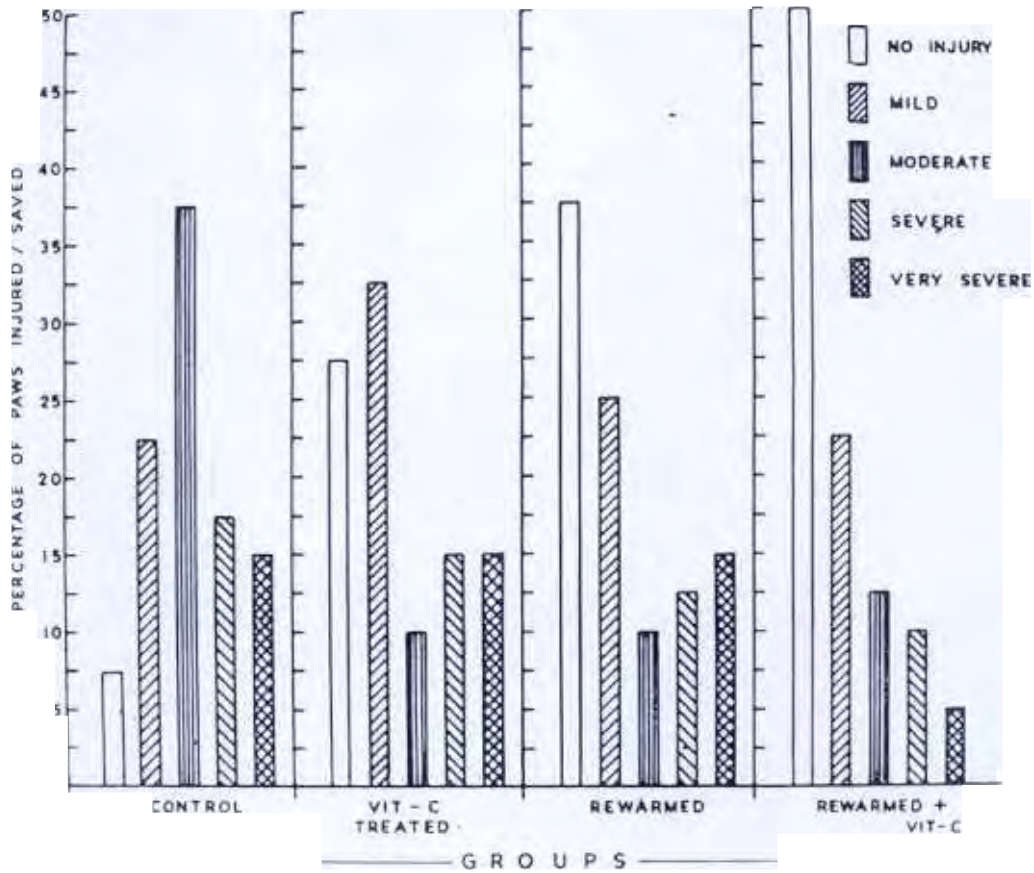

Figure 5. Percentage distribution of the degree of cold injury in various groups of animals of treatment series.

The approach for the prevention of cold injury has been made on the assumption that an increase in cold tolerance may rise the extremity bloodflow and prevent statis of blood and blocking of small vessels on exposure to cold. It has been shown that administration of Vit C at large doses increase the cold tolerance[15]. The result of present experiments showed that Vit C administration for a short period of seven days was of no benefit. However, prolonged administration of large doses of Vit C for 21 days showed significant improvement in reducing the incidence of frostbite as well as less fall in rectal temperature during cold exposure. The second set of Series I experiments, where the same animal was used as its own control, confirm the result of the first set. Even though the exact mechanism is not well understood, the beneficial effect of long term administration of Vit C therapy might be due to the improvement in the cold tolerance because of increased metabolism[15], and this might have kept the tissues warmer by increasing the extremity bloodflow. The effect may be through thyroid hormone as postulated by Des Marias[16]. Duggal[17] has also observed that Vit C in large dose was beneficial to laboratory animals including monkeys when these are exposed to cold environment. The large dose of Vit C has also been proved effective in delaying hypothermia[18]. The observation in rats by Dasgupta, *et al*[19] supports our findings. Thus it appears that administration of high dose of Vit C for a prolonged period before cold exposure is beneficial for significant improvement in cold tolerance and reduction in the incidence of frostbite in animals.

Physical well being, proper insulating footwear, nutritious food and good hygiene are some of the important measures for the prevention of frostbite. Inspite of best efforts, application of all these may be difficult and inadequate under field conditions during war and accidental situations such as sudden storm, or prolonged out door exposure due to unforeseen reasons. Under such circumstances possibility of getting cold injuries could not be completely avoided.

Further, frostbite once occured cannot be treated satisfactorily, particularly when there is a delay in starting the therapy. And in field situations, there is always delay to evacuate the frostbite victims from the field to the rear areas in the hospital due to serious logistic problems. The line of treatment advocated[4-7] does not take into account the immediate treatment required for such cases. The treatment will be most effective, if it could be given immediately on recognition of the injury at the site of occurrence itself. In Series II experiments, large dose of Vit C therapy and rapid rewarming of the exposed parts for 30 minutes at water bath maintained close to body temperature (37 °C) immediately after exposure resulted in significant reduction in the extent of tissue damage. The optimum temperature of rewarming is close to the body temperature which has already been demonstrated by Malhotra and Mathew[20]. Rapid rewarming possibly acts by restoring bloodflow rapidly so that there is inadequate time for plasma to be lost and the red cells to sludge during passage of blood through tissues[11,21]. Further, with rapid rewarming, the area of circulatory arrest is smaller to that after slow rewarming and this is no doubt, responsible for the increased tissue survival[22]. Moreover administration of large dose of Vit C might have helped in restoring the intercellular substances in the normal form throughout the body which is a major physiological function of ascorbic acid. This includes formation of collagen and intercellular cement substance between the cells, formation of bone matrix and formation of tooth dentin. Without ascorbic acid, the collagen formed is defective and weak. Hence, Vit C is essential for growth of subcutaneous tissues, cartilage bone and teeth[23]. Combination of rapid rewarming and administration of large dose of Vit C immediately after exposure to frostbite condition showed additional benefit. Thus, from this study it can be presumed that prolonged Vit C therapy in large dose preceded by rapid rewarming at water-bath close to body temperature (37 °C) is suitable as an immediate treatment for cold injuries in animals like rat. The same may be said on human subjects even though this needs further work to confirm it. The treatment may be most effective if administered immediately on recognition of the injury.

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