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Evaluation of Anti-Oxidant Effect of Oral β-Carotene and Topical Lycopene on Burns Wound Induced Rats

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ABSTRACT

This study attempt to evaluate the antioxidant activities of oral β-Carotene and topical lycopene on burn wounds healing in rat model, partial thickness thermal burn wounds were inflicted in all groups of animals. Group I was assigned as Control, Group II was received beta carotene (oral), Group III was received Lycopene(topical) and Group IV was received beta carotene orally plus topically applied Lycopene. The parameters observed were epithelization period, percentage of wound contraction and TBARS levels and SOD levels as indicative of the process of healing. The mean values of the epithelization period was significantly reduced in beta carotene plus lycopene topically applied group of rats as compared to control group of rats. The mean values of the epithelization period was slightly decrease in beta carotene treated rats and topically applied lycopene treated rats when compared to control group of rats. The percentage of wound contraction was significantly increased in beta carotene plus topically applied lycopene group of rats when compared to control group of rats. The percentage of wound contraction was slightly increased in beta carotene treated rats and as well as only topically applied lycopene when compared to control group of rats. The serum TBARS levels were significantly increased in burn induced control group of rats (group I) compared to the normal or healthy rats. Administration of beta carotene plus topically applied lycopene rats were significantly decreased the levels of serum TBARS as compared with control rats (groupI). The serum SOD levels were significantly reduced in burn induced rats(group I) as compared with healthy rats. Administration of beta carotene plus topically applied lycopene in rats were significantly increase the SOD levels on 21st day in comparision with burn induced group of rats (group I). The conclusion clearly shows the beneficial effect of the beta carotene plus topically applied lycopene treatment to enhance the wound healing in burn.

Key words: Epithelization, Beta carotene, Lycopene, Wound contraction

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INTRODUCTION

Burn is a post traumatic inflammatory disease accompanied by both local and distant effects leading to intense inflammation, tissue damage, and infection. In the pathophysiology of burns, at the molecular level, both complement activation and intra-vascular stimulation of neutrophils result in the production of cytotoxic reactive oxygen species (ROS) which has been implicated in emergence of local and systemic oxidant changes as manifested by increased oxygen free radical activity[1] and lipid peroxidation in animal burn models and also in burned humans[2] Oxidative stress may also contribute to secondary tissue damage and impaired immune function in patients after burn injury. The best documented antioxidant action of carotenoids is their ability to quench singlet oxygen. This results in an excited carotenoid, which has the ability to dissipate newly acquired energy through a series of rotational and vibrational interactions with the solvent, thus regenerating the original unexcited carotenoid, which can be reused for further cycles of singlet oxygen quenching. The quenching activity of a carotenoid mainly depends on the number of conjugated double bonds of the molecule and is influenced to a lesser extent by carotenoid end groups (cyclic or acyclic) or the nature of substituents in carotenoids containing cyclic end groups.

MATERIALS AND METHODS

Drugs and chemicals

β-Carotene, Lycopene (Biorigine Life Sciences P. Ltd., Pondicherry), TBA, Tris HCl buffer, Malondialdehyde, Ethanol, Ether, Methanol, Chloroform, Phosphate buffer, Sodium pyrophosphate buffer, Phenazine methosulphate, Nitroblue tetrazolium, TCA, NADH, Glacial Acetic acid, n-butanol(S.D. Fine chemicals, Mumbai, India), Thiopentone(Ranbaxy, Guragon, India).All other chemicals were analytical grade.

Animals

A total number of 24 healthy(180-220gm)Sprague Dawley (SD) rats were divided into 4 groups of 6 rats ,of either sex aged 10 -12 weeks were purchased from Kings Institute Guindy Chennai. They were housed under controlled conditions of room Temperature was maintained at 22°C ±2°C and 50% to 70% relative humidity with alternate12-hour light-dark cycles with proper ventilation as per the guidelines of Indian National Science Academy, New Delhi, India. Rats were housed individually in polypropelene cages containing sterile paddy husk(procured locally) as bedding throughout the experiment and had free access to sterile food and portable water ad libitum. Animals were kept under fasting overnight and weighed before the experiment. The

Research project was approved by Institutional Animal Ethics Committee and (CPCSEA – Committee for the Purpose and Control of Supervision on Experimental Animals).

Experimental design and Drug administration

Rats (24) were divided randomly into 4 groups of 6 rats each. Group I was assigned as Control, Group II was received beta carotene orally(30mg/kg b.wt/oral/day), Group III was received Lycopene topically and Group IV was received beta carotene (30mg/kg b.wt/oral/day) plus topically applied Lycopene. All the drugs were given daily for 21 days or till complete epithelization whichever was earlier.

Wound model

A partial thickness burn wound model was employed as per Bairy et al. After overnight starvation animals were anesthetized by intra-peritoneal (i.p.) injection of thiopentone (25 mg/kg), the dorsal surface of the rat was depilated by shaving, and the underlying skin was cleaned with 70% ethanol. Partial thickness burn wounds was inflicted in all groups of animal by pouring hot molten wax at 80°C into a metal cylinder with a 2 cm diameter circular opening, placed on the back of the animal for few seconds. Animals were allowed to recover from anaesthesia, fluid replacement was given with Ringer Lactate and housed individually in sterile cage and followed all norms of good laboratory practice in caring the animals.

Assessment of burn healing

Animals were inspected every alternate day and the healing was assessed based on physical parameters namely wound contraction and epithelization.

Epithelization period

It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind.

Wound contraction

It was noted by following the progressive changes in wound area plan metrically, excluding the day of the wounding. The size of the wounds would be traced on a transparent paper every two days, throughout the monitoring period. The tracing were then transferred to 1 mm^2 graph sheet, from which the wound surface area were evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial area of the wound,4.7cm^2 , as 100% using the formula below:

Percentage of Wound Contraction = Initial wound size - specific day wound size / Initial wound size x 100

Evaluation of TBARS and SOD

After the fall of eschar, rats were sacrificed by administering intravenous thiopentone sodium. A known amount of the serum was homogenized with 2.5 ml of ethanol-ether mixture (3:1 v/v) and digested for about two hrs at 60-65ºC and the supernatant was collected. 3 ml ethanol-ether mixture was added to the residue, digested further for a period of two hrs at 60-65ºC and the supernatant was collected.1ml of chloroform-methanol mixture (1:1 v/v) was then added to the residue. It was again digested for one hour at 50-55°C and the supernatant was collected. The supernatant was pooled and made up to a specified volume. This lipid extract was finally used for the estimation of TBARS levels. Another portion of serum was homogenated with phosphate buffer saline and used for the estimation of SOD.

Statistical analysis

Results were expressed as mean ± SE of 6 rats in each group. One ways analysis of variance (ANOVA) with Scheffe's multiple comparisons test were used to determine the statistical significance. Significance level was fixed at 0.05.

RESULTS AND DISCUSSION

The mean values of the epithelization period was significantly reduced in beta carotene plus lycopene topically applied group of rats (12.41± 0.165 days) as compared to control group of rats (21.44± 0.42 days). The mean values of the epithelization period was slightly decease in beta carotene treated rats (17.00± 0.42 days) and topically applied lycopene treated rats(18.00± 0.64 days) when compared to control group of rats (21.44± 0.42 days).

The percentage of wound contraction was significantly increased in beta carotene plus topically applied lycopene group of rats when compared to control group of rats. The percentage of wound contraction was slightly increased in beta carotene treated rats (group II) and as well as only topically applied lycopene (group III) when compared to control group of rats (group I).

The serum TBARS levels were significantly increased in burn induced control group of rats (group I) compared to the normal or healthy rats. Administration of beta carotene rats were significant decrease the levels of serum TBARS as compared with control rats (groupI). A topically applied lycopene treated rats also showed decreased level of serum TBARS level. Treatment of beta caroteneplus topically applied lycopene rats were significant decrease the levels of serum TBARS as compared with control rats (groupI).

The serum SOD levels were significantly reduced in burn induced rats(group I) as compared with healthy rats. Administration of beta carotene group of rats serum SOD levels were

significantly increased on 21st day in comparison with burn induced group of rats (group I).The serum SOD levels were significantly increased in beta carotene plus topically applied lycopene treated group of rats on 7th day of post burn as compared to burn group of rats(group I) as well as only topically applied lycopene treated rats (group III).

Table 1 Effect of β-carotene & Topical Lycopene on burn wound contraction in rats

Values are Mean ± SE

S No.	Group	Wound contraction (%) 3rd day	7th day	11th day	15th day	19th day
1.	Group I(burned)	3.71± 0.035	10.50± 0.15	22.07± 0.31	41.36± 0.38	56.67± 0.24
2.	Group II (burned + β-carotene Oral)	10.21± 0.031a*	27.25± 0.071a*	39.06± 0.041a*	55.02± 0.18a**	77.32± 0.27a*
3.	Group III (burned +Topical Lycopene)	9.34± 0.049a**	16.23± 0.031a*	36.47± 0.12a*	51.21± 0.025a*	68.57± 0.11a*
4.	Group IV (burned + β-carotene plusTopical lycopene)	24.24± 0.15a*	42.42± 0.14a*	68.22± 0.24a*	79.39± 0.15a*	93.65± 0.15a**

P value *<0.001** < 0.05 a→ group 1 compared with group II, III, IV

Table 2 Effect of β-carotene & Topical Lycopene on TBARS level in burn wound induced rats Values are Mean ± SE

S No.	Group	Serum TBARS level (MDA nmol/ml) 3rd day	7th day	11th day	15th day	19th day
1.	Group I (burned)	6.57± 0.12	6.69± 0.019	6.41± 0.15	5.81± 0.067	5.11± 0.014
2.	Group II (burned +β-carotene oral)	5.18± 0.021a*	4.90± 0.025a*	4.16± 0.019a*	3.14± 0.013a*	2.32± 0.030a*
3.	Group III (burned +Topical Lycopene)	5.65± 0.036a**	5.10± 0.026a*	4.44± 0.074a*	3.57± 0.016a**	2.87± 0.024a*
4.	Group IV (burned +β-carotene plus Topical Lycopene)	4.69± 0.018a*	3.84± 0.047a**	1.97± 0.030a*	1.18± 0.016a*	0.95± 0.015a*

P value *<0.001 ** < 0.05 a→ group 1 compared with group II, III, IV

Table 3 Effect of β-carotene & Topical Lycopene on SOD levels in burn wound induced rats Values are Mean ± SE

S No.	Group	Superoxide dismutase (SOD) unit/ml 3rd day	7th day	11th day	15th day	19th day
1.	Group I (burned)	3.82± 0.009	4.11± 0.030	4.24± 0.032	4.35± 0.020	4.79± 0.030
2.	Group II	4.71±	4.93±	5.13±	5.29±	5.70±

	(burned +β-carotene Oral)	0.019a*	0.016a*	0.017a**	0.021a*	0.015a*
3.	Group III (burned +Topical Lycopene)	4.11± 0.020a*	4.25± 0.021a*	4.50± 0.057a*	4.52± 0.067a*	4.67± 0.023a*
4.	Group IV (burned +β-carotene plus Topical Lycopene)	6.31± 0.021a*	6.57± 0.028a**	6.79± 0.028a*	7.15± 0.029a**	7.34± 0.019a*

P value *<0.001 ** < 0.05 a→ group 1 compared with group II, III, IV

In the present study results show that beta carotene plus topically applied lycopene increased the epitheliazation period. This effect may be due to increasing fibroblast proliferation and maturation of collagen content[3] The results shows that enhanced the percentage of wound contractionin beta carotene plus topically applied lycopene treated rats. This effect may be due to improved the quality and vascularity of granulation tissue and decreaseing collagenase activity[4].

The present investigation serum TBARS levels were increased in burn induced rats. This effect due to the excessive activity of free radical that results oxidative stress state during burn injury[5]. This study shows that beta carotene along treated rats slightly reduction of serum TBARS levels. This may be due to contrasting effect on rates on peroxidation that relate to their membrane lipid interactions[6] Beta carotene is also scavengers of peroxyl radicals especially at low oxygen tension[7].

Topically applied lycopene serum TBARS levels were significantly reduced. This effect may be due to lycopene (11 conjugated and 2 non conjugated double bond) is among the most efficient singlet oxygen quenchers of the natural carotenoids[8]. This study shows that the beta carotene plus topically applied lycopene treated rats were highly significantly decrease the levels of serum TBARS. This may be due to the prevention of lipid peroxidation by carotenoids (beta carotene plus lycopene) has been suggested to be mainly via singlet oxygen quenching[9].

SOD is a natural antioxidant enzyme playing an important role in self-defense mechanisms of cells against oxidative stress. It converts the superoxide anion to less bioactive hydrogen peroxide and oxygen molecules. Researchers have reported that SOD activity in plasma steadily decreases after burns because of increased consumption of activated SOD[10,11].

The present investigation serum SOD levels were decreased in burn induced rats. This decrease may be related to the consumption of activated enzyme against oxidative stress. This study shows that beta carotene along treated rats slightly increase of serum SOD levels. This may be due to scavenges free oxygen radicals or decreases MPO activity in neutrophils. This study

shows that the beta carotene plus topically applied lycopene treated rats were highly significant increase the levels of serum SOD. This may be due to directly increase the antioxidant enzyme activity and prevent the inhibition of the activities of these enzymes.

CONCLUSION

The results obtained in this study clearly shows the beneficial effect of the beta carotene plus topically applied lycopene treatment to enhance the wound healing in burn. β-carotene and others carotenoids have antioxidant properties, but the antioxidant capability is variable depending on the *in vitro* system used. The antioxidant activity of these compounds can shift into a prooxidant effect, depending on such factors as oxygen tension or carotenoid concentration. Mixtures of carotenoids alone or in association with others antioxidants can increase their activity against lipid peroxidation Finally our study shows that it is highly recommendable to add antioxidant such as carotenoids for treatment of burn wound healing.

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