Original Article

Cardiovascular and Osteoporosis Protection at Menopause with Lycopene: A Placebo-Controlled Double-Blind Randomized Clinical Trial

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ABSTRACT

Objective: The effect of lycopene (LycoRed) supplementation was evaluated in healthy postmenopausal women by biochemical markers for cardiovascular protection and osteoporosis protection. **Study Settings and Design:** This was a multi-centric placebo-controlled double-blind randomized clinical trial that screened 198 postmenopausal women at 21 centers across 12 cities in India. Levels of lycopene, lipid profile, high-risk C-reactive protein, and bone turnover markers: amino-terminal propeptide of Type I collagen (P1NP) and C-terminal telopeptide of Type I collagen (β-CTx) were measured at baseline and 6 months postsupplementation with LycoRed or placebo. **Interventions:** The study was completed with 57 of the 100 women on LycoRed 8 mg (antioxidant potency is equivalent to 24 mg of lycopene) and 43 placebos for 6 months by randomization. **Main Outcome Measures:** Rise in serum lycopene and effect of serum lycopene on surrogate markers of cardiovascular health and bone health. **Results:** LycoRed supplementation increases lycopene levels and P1NP and nonsignificant fall in β-CTx levels in healthy postmenopausal women. **Conclusions:** Lycopene supplementation in Indian menopausal women may confer protection from osteoporosis as shown by the directional change in the surrogate biochemical markers. This study can form a basis for larger studies with different doses to understand the effect of lycopene to prevent and act as adjuvant treatment on clinical endpoints for cardiovascular disease (CVD) and bone health.

KEYWORDS: *Bone turnover markers, cardiovascular disease, lycopene, osteoporosis, postmenopause*

INTRODUCTION

India, with a population of 1.2 billion people, accounts for more than 100 million, over 50 years of age.[1] According to the latest World Health Organization (2018),[2] the life expectancy in India for a female is 70.3 years, expected to increase to 77 years by 2050. Noncommunicable diseases account for 60% of the total deaths in India. From the available Indian data, it is hypothesized that the early age of menopause predisposes a woman to chronic health disorders a decade earlier than a Caucasian woman.[3] It is reported that osteoporotic fractures and cardiovascular disease (CVD) occur 10–20 years earlier in Indians compared to Caucasians.[4] There is an enhanced bone turnover as suggested by the increase in both bone resorption and bone formation markers and a net bone loss at the rate of 1%–5%/year.[5] Menopause is associated with a decrease in estrogen leading to a pro-atherogenic state. There is increasing evidence that women who experience menopause at an age well before the median age of natural menopause are at increased risk of mortality and morbidity.[6]

The biomarkers of CVD, lipid profile, and high risk C-Reactive Protein (hs-CRP) test play an important

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role in predicting risk over years. Bone turnover markers (BTMs) indicate the rate of bone turnover and are used for monitoring osteoporosis treatment and compliance. Combinations of demographic and BTM predict some 30 ± 40% of the variance in bone loss rates in untreated postmenopausal women.[7] The international osteoporosis foundation and the international federation of clinical chemistry in 2010 chose PINP and β-CTx as preferred biomarkers in osteoporosis.[8] Aging, together with reduced concentrations of estrogen results in increased oxidative stress at menopause transition.[9]

Phytochemicals present in fruits and vegetables play a major role in noncommunicable disease management and serum carotenoids are used as biomarkers for fruit and vegetable consumption.[10-13] Lycopene is the most predominant carotenoid in human plasma with a high antioxidant property. Studies have shown lycopene to be thrice as potent as β-carotene and ten times as potent as α-tocopherol concerning its singlet oxygen quenching ability.[14] The National Family Health Survey-3 India,[15] from the self-reported data on fruit and vegetable consumption, showed that half of the population in its survey consumed zero or only 1 serving of fruit in a week versus the advocated requirement of one cup or hundred grams of fruit and three hundred grams vegetable per day.[16]

Increased oxidative stress in menopausal women plays an important role in atherosclerosis as oxidative modification of LDL by the free radicals enhances their atherogenicity. Lycopene inhibits the formation of osteoclasts and associated bone resorption and it stimulates proliferation and differentiation of osteoblasts by its antioxidant and biological activities, interacting synergistically with vitamin D on cell proliferation, differentiation, and cell cycle progression.[17]

Preclinical, epidemiological, and clinical studies have shown the role of lycopene as a strong antioxidant for the prevention of a variety of cancers and chronic diseases.[18,19] The rationale of this double-blind randomized controlled trial (RCT) was planned to look at the effects of lycopene supplementation on surrogate markers of CVD and bone health in healthy Indian women menopause. Based on dietary and supplementation studies on lycopene, the hypothesis was that LycoRed supplementation will significantly improve the levels of serum lycopene with a beneficial effect on CV and osteoporosis risk.

Methods

Participants and enrolment

The study design is an interventional parallel-group double-blind superiority RCT (phase IV).

This multicentric study screened 198 women and recruited 176 postmenopausal women at 21 centers across 12 cities in India. The enrolment period was 12 weeks after confirming eligibility and obtaining informed consent. The inclusion criteria for the study were women between 40 and 55 years of age with natural menopause characterized by cessation of menses more than 1 year, or in case of surgical menopause with follicle-stimulating hormone level ≥30 U/L. Women with a history of smoking, and chronic medical disorders like diabetes, hypertension or heart disease, dyslipidemia, thyroid dysfunction were ineligible to participate in the study. Women taking lipid-lowering agents, therapy for osteoporosis, antioxidants, supplements, or those who had used hormone replacement therapy in the past 6 months were also excluded from the study.

The mean age of the enrolled women was 49.8 years. A detailed history with a special emphasis on dietary habits, clinical examination, and baseline investigations (complete hemogram and fasting blood glucose) were performed to exclude significant medical illness. The menopausal symptoms and the severity were recorded using the menopause rating scale.[20]

An overnight fasting blood sample was drawn to do a lipid profile including total cholesterol (Chol), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very LDL, and triglycerides (TG), lycopene, hsCRP, P1NP, β CTx-1 for 176 women. These tests were repeated after 6 months for the 100 women who completed the study. Blood lycopene was extracted by using absolute alcohol and petroleum ether and analyzed by high-performance liquid chromatography. Lycopene level was measured as a single peak containing all trans-and cis-isomers using an absolute detector set at 472 nm. An external standard of lycopene (Sigma Chemical Co.) was used as a reference standard. Total lipid profiles, hsCRP, were analyzed using commercially available ELISA kits as per the manufacturers' protocol. P1NP and β-CTx-1was done with CLIA-COBAS 411. The reference range for postmenopausal women for β-CTx-1 is 0.010–1.01 ng/ml and for P1NP is 16.27–73.87 ng/ml. The samples were processed at a centralized Super Religare Laboratories in Mumbai, India.

Standard protocol approvals, registrations, and patient consents

The study was registered with the Clinical Trials Registry of India (CTRI/2010/091/000089) and Ethical Committee clearance for the study was taken from Independent Ethical Committee, Hyderabad. A patient information sheet was handed over and Counselling was done. The participant's informed written consent to be a part of the trial was taken in their local language.

To ensure compliance and a low dropout rate, the investigators were advised to enroll relatives, friends, and patients known to them and maybe trusted to follow the trial as required.

Sample size

The impact of lycopene supplementation on P1NP levels was considered as the main outcome measure for sample size calculation. In the absence of reliable data regarding improvement in P1NP levels, a moderate effect size of 0.6 was assumed. With this effect size, a sample of 44 per group was required to achieve a power of 80% allowing for a 5% Type I error. Considering 20% lost to follow-up, the sample size was inflated to 105 (±5).

Randomization

The randomization schedule was determined by the bio-statistician, using blocks of unequal length with an allocation of 1:1 in each arm. The randomization schedule was maintained at the study central office. Each center was given the drug (verum/placebo) in three box cartons containing 24 strips (each strip had 10 soft gels). The investigator at the participating center would contact the study central office in case of any adverse effects noticed to decode the drug (through the bio-statistician).

The intervention and the control arm: Each soft gel of LycoRed has 2 mg as lycopene along with the associated phytonutrients suspended in tomato oleoresin oil. In addition, it contains 7.5 mg of zinc and 35 µg of selenium. A similar-looking identical placebo with 7.5 mg of zinc and 35 µg of selenium without the active ingredient. LycoRed in the dosage of two capsules of 2 mg each were given twice daily after meals, i.e., 8 mg/day (antioxidant potency is equivalent to 24 mg of lycopene) or an identical placebo given as two capsules for 6 months by randomization. No diet restriction was advised to the women. A CONSORT flow diagram is shown in Figure 1.

Main outcome measures

The primary outcomes measured were change in serum lycopene levels, HDL, LDL, TG, P1NP, β-CTx (difference between baseline and postsupplementation values) before and after supplementation.

Statistical analysis

The categorical data were represented in the form of frequencies and proportions. Continuous data were represented as mean and standard deviation (SD). Student's *t*-test was used for quantitative data to test the significance between the two groups. All the charts were created on MS Office. Data were analyzed at an aggregate level through Python.

Results

A total of 198 women were assessed for eligibility, where 22 were excluded pertaining to the criteria. The remaining 176 women were screened out of which 40 did not meet the set criteria and 28 declined to participate. Finally, 108 women were divided into the study group (60) and the placebo group (48). Three participants from the study group and five from the control group were lost to follow-up. Analysis was performed on 57 in the study group and 43 women in the placebo group for cardiac and bone markers [Figure 1].

Demographics of the participants are stated in Table 1. Baseline levels of serum lycopene, cardiac and bone markers are given in Table 2. Based on the tables, baseline profiles of participants were similar.

After 6 months of lycopene supplementation, improvement in blood lycopene levels was significantly higher in the study group as compared to the placebo (mean 71.0 [SD 217.16] improvement in LycoRed versus mean –94.1 (SD 195.8) deterioration in placebo, $P = 0.003$] [Figure 2].

Table 1. Demographics

	Overall	Study Group	Placebo
Age (years), mean (SD)	49.8 (4.0)	49.8 (3.9)	49.7 (4.2)
Occupation: n(%)			
House Wife	53 (53%)	33 (58%)	20 (47%)
Working	47 (47%)	24 (42%)	23 (53%)
Residence: n(%)			
Urban	93 (93%)	52 (91%)	41 (95%)
Rural	7 (7%)	5 (9%)	2 (5%)
Diet: n(%)			
Vegetarian	55 (55%)	34 (60%)	21 (49%)
Non-Vegetarian	39 (39%)	21 (37%)	18 (42%)

SD: Standard deviation

Table 2: Baseline levels - Cardiac and Bone markers (pre-supplementation)

Averages (95% CI)	Study Group	Placebo
Lycopene (ng/mL)	191.7 (140 to 243)	212.5 (147 to 278)
Cardiac Markers		
HDL(mg/dL)	48.0 (45 to 51)	49.4 (46.5 to 52.3)
LDL(mg/dL)	123.7 (117 to 131)	121.7 (113 to 130)
Triglycerides(mg/dL)	124.1 (107 to 141)	121.5 (101 to 142)
hs-CRP(mg/L)	3.3 (2.6 to 4.1)	4.4 (2.1 to 6.7)
Bone Markers		
CTx-1 (ng/mL)	0.5 (0.3 to 0.7)	0.3 (0.3 to 0.4)
P1NP (ng/mL)	70.3 (62.2 to 78.4)	50.5 (45.6 to 55.4)

HDL: High-density lipoproteins, LDL: Low-density lipoproteins, hs-CRP: High-risk C reactive protein, CTx: C-terminal telopeptide of type 1 collagen, CI: Confidence interval, P1NP: Procollagen type I N propeptide

Further evaluation has been done on lycopene supplementation on cardiac markers. The change in HDL in 6 months was –0.75 mg/dL (SD 9.5) in the study group and –0.30 mg/dL (SD 6.7) in the placebo group. LDL average differences in the study group were 3.75 mg/dL (SD 32.2) and placebo group were 2.84 mg/dL (SD 22.1). No statistical significance was noted between the study group and placebo group on change in HDL and LDL (Baseline and post supplementation) [Figure 3]. The change in TG was 28.42 mg/dL (SD 79.0) in the study group and –3.49 mg/dL (SD 65.5) in the placebo group. This difference was significantly higher ($P < 0.05$) in the study group as compared to the placebo group [Figure 3].

The improvement in hs-CRP of the study group was not significant compared to the placebo group, the change in values over 6 months were –0.73 mg/L (SD 2.3) in the study group and –0.51 mg/L (SD 7.3) in the placebo group [Figure 3]. Within the study group, there was a significant decrease in hs-CRP postsupplementation, which was not significant in the placebo group.

The change in β-CTx-1 over the 6 months was –0.13 ng/mL (SD 0.7) in the study group and –0.04 ng/mL (SD 0.1) in the placebo group, the difference being not statistically significant (study group versus placebo group). The improvement in the study group was significant ($P < 0.1$) as compared to the placebo group in P1NP, the change in values over 6 months were –9.70 ng/mL (SD 25.0) in the study group and –2.96 ng/mL (SD 10.2) in the placebo group [Figure 4]. Changes in BTM greater than about 20% for PINP and about 25% to 30% for CTX were significant and greater than least significant change LSC. Change in bone markers over the 6 months using the LSC, a difference noted in the study group versus the placebo group. About 46% of the women in the study group had a drop less than the LSC in β-CTx as compared to 33% in the placebo group. While 37% of the women in the study group had a drop less than the LSC in P1NP as compared to 23% in the placebo group. The average LSC percentage for both the bone markers showed a negative correlation with supplementation of LycoRed [Figure 5].

Discussion

In the present study, serum lycopene levels have increased significantly after LycoRed supplementation [Figure 2]. There was also a significant drop in blood lycopene levels in the placebo group. This can be attributed to the dietary habits of the respective individuals. In addition, an individual's response to lycopene varies and it has been shown that a weak response to dietary lycopene is based on the presence of other single nucleotide Polymorphisms in the BCO1 gene.[21]

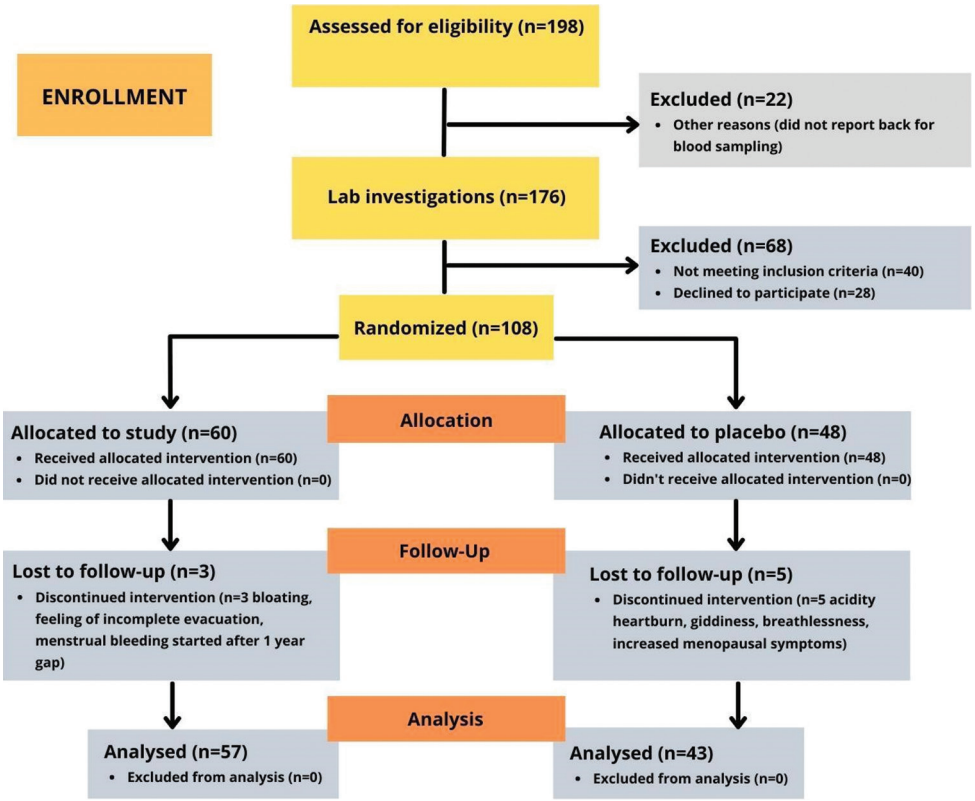

Figure 1: CONSORT flowchart of the progress through the phases of a randomized trial

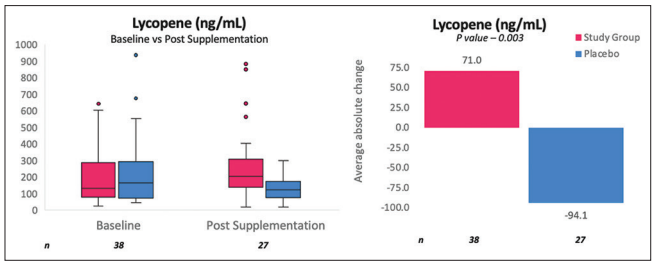

Figure 2: Impact of lycopene supplementation on serum lycopene levels

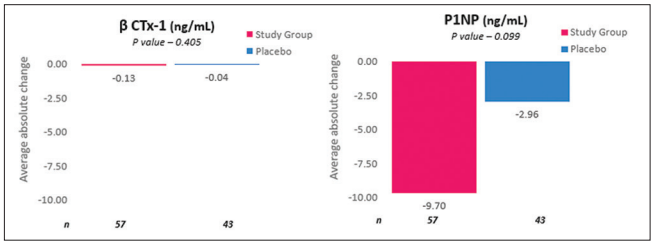

Figure 4: Impact of lycopene supplementation on Bone markers

Serum cholesterol and oxidative stress levels are important risk factors for CVD.[22] The lycopene levels naturally decrease with age at menopause unless supplemented by diet. Lycopene is believed to act as an anti-inflammatory agent and mediate its action through cytokines and transcription factors and thus decrease hs-CRP levels.[23] Lycopene blocks gene expression of pro-inflammatory genes IL-6, Il-1 β, and MCP1-1 by blocking the NF-kB signalling.[24] A negative correlation has been observed between hs-CRP and lycopene levels in the blood of menopausal women. The results were concurrent with the study conducted by Kim *et al*.,[25] i.e., after 15 mg/day lycopene supplementation, for 8 weeks, reported a decrease of systolic blood pressure and high sensitivity CRP. LycoRed supplementation for 6 months increases lycopene and reduces hs-CRP levels significantly thereby providing a protective shield to menopausal women from cardiovascular risk [Figure 3]. A systematic review compiled data from RCTs and further performed a meta-analysis, finding that overall carotenoid supplementation significantly reduced CRP and IL-6 levels.[26] Another study by Chauhan AP *et al*.[27] found a significant effect of lycopene intervention on the levels of CRP. A pilot study in heart failure patients showed that lycopene intervention decreased CRP level significantly in women but not in men.[28]

HDL is a scavenger of LDL and its increase positively correlates with decreased CVD risk. In our study, there is no significant change in HDL or LDL in both LycoRed and placebo groups [Figure 3]. There was a significant increase in TG levels in the LycoRed group and no change was noted in the placebo group [Figure 3]. Our study was concurrent with the Petyaev IM *et al*. study

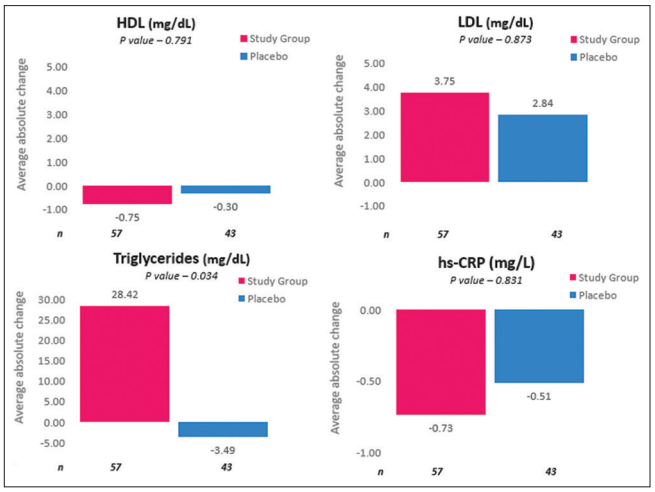

Figure 3: Impact of lycopene supplementation on cardiac markers

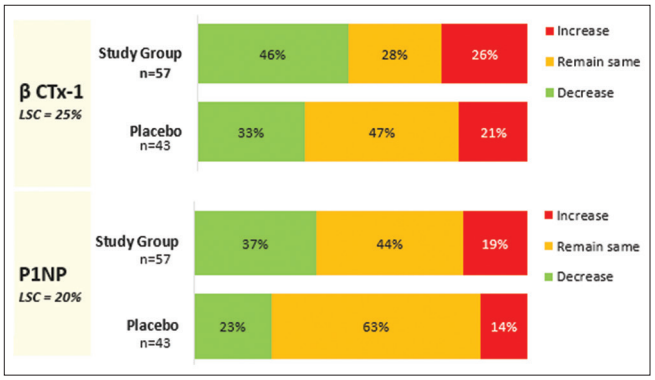

Figure 5: Least Significant Change (LSC) in bone markers post lycopene supplementation

in which supplementation with two kinds of lycopene did not significantly affect parameters of LDL and HDL.[29] A meta-analysis by Ried and Fakler showed that lycopene is effective in reducing total cholesterol and LDL cholesterol in serum if taken in doses higher than 25 mg daily.[30] Petyaev *et al*. conducted a study on patients aged 45–73 years which showed lowered LDL levels after supplementation with lycopene.[29] TG have shown a significant rise which was similar to the study conducted by Renu Misra *et al*.,[31] which showed an increase in HDL and TG. An increase in the TG levels can also be attributed to the genetic predisposition and relative increase of the abdominal fat amount and insulin resistance during menopause.[32,33] Perhaps the dose, duration of supplementation and baseline lycopene levels may show beneficial effects.

Low levels of serum lycopene have been reported in postmenopausal women with osteoporosis as compared to the healthier ones.[34] The β-CTx levels showed no significant change in both the groups and a statistically significant drop in P1NP levels post Lycopene supplementation [Figure 4]. The trend towards bone

protection is indicated by an average drop in the LSC in β-CTx by 46% in the women on the LycoRed versus 33% on the placebo and 37% of the women on LycoRed had a drop less than the LSC in P1NP as compared to 23% in the placebo. The differential response as seen in Figure 5 depicts the individualized response and reflects the nonresponders depending on the genetic and environmental factors. A study conducted by Ardawi MS *et al*.,[35] concluded that an increase in dietary intake of lycopene showed higher serum lycopene and has positive effects on bone health, increases in bone formation (P1NP) by 13.0%–32.1%; and decreases in bone resorption (β-CTx-1) by 22.8–27.5%. Similarly, another study done in the University of Aberdeen, Scotland showed that the benefits of lycopene may accrue over the lifespan, but short-term benefits are less likely to arise other than in populations with high bone turnover (for example, in menopausal women) where there is greater potential to see measurable benefits. In contrast to the above studies, a pilot conducted by Russo C *et al*.,[36] showed no significant change in the CTX levels postsupplementation with lycopene-derived food items.

Strengths and limitations of the study

This paper is the first clinical double-blind placebo-controlled RCT with the use of validated markers of bone health. The strength of this clinical study comes from fairly good internal validity and to an extent external validity. The study group enrolled a diverse population from all over India. The challenge was shortlisting healthy women at menopause who were not on supplements. The limitations include an imbalance of the sample size at the overall level even though 1:1 randomization and allocation was ensured at the site level. The baseline nutritional data was captured and the diet and physical activity were not considered in the follow-up visits.

Conclusions

This study gives a direction on the benefits of lycopene supplementation in Indian menopausal women to maintain cardiac and bone health. No side effects or discomfort were reported during the study. The effects of different doses and duration of lycopene for prevention and as an adjuvant along with treatment for specific indications need to be studied.

In the prevention of such chronic diseases, there is no single magical ingredient, it is the combined roles of genetics, micronutrition, macro nutrition, antioxidants, and functional foods in the background of lifespan factors like physical activity, exercise, sleep, and environment. The role of cost-effective phytochemicals as long-term supplements at vulnerable phases of life needs to have synergistic effects of bioactive antioxidants and other food ingredients in the background of the heterogenous response of each individual. This may bring about new perspectives in developing new functional foods for prevention and therapy.

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LycoRed soft gels containing Lyc-O-Mato and identical placebo used in the study were provided by Jagsonpal Pharmaceuticals Limited, New Delhi. LycoRed is a US world-process-patented, US FDA GRAS approved product of LycoRed Ltd., Beer-Sheva, Israel. The authors remained free of bias in conducting the study.

Conflicts of interest

There are no conflicts of interest.

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