Feeding of 0.33% cholesterol rich diet to rabbits for 22.4 weeks was associated with a substantial hyperlipidemia. The efficacy of TRF or Tocomin in the prevention of experimental hyperlipidemia in rabbits has been shown by feeding of 16.2 mg% TRF or 6.97 mg% Tocomin together with a cholesterol rich diet for 22.4 weeks. TRF and Tocomin significantly prevented the increase in plasma TG, TC, VLDL-C, and LDL-C levels in comparison to rabbits fed cholesterol rich diet alone. However, elevated plasma HDL-C, HDL_{3}-C and HDL_{2}-C levels were not reduced significantly in TRF and Tocomin treated rabbits. Both Tocomin and TRF caused a substantial improvement in the ratios of HDL-C/TC, HDL-C/ LDL-C, TC/ HDL-C and LDL-C/ HDL-C, in comparison to ratio values of hyperlipidemic control indicating the initiation of normalization process of lipid parameters.

In response to oxidative stress, evoked in experimental hyperlipidemia in rabbits, as reflected by increased formation of plasma lipid peroxides, higher base line levels of diene conjugation (BDC) of LDL, as modified in vivo, increased rates of conjugated diene formation in LDL and decrease in lag phase time of LDL oxidation in vitro, was substantially blocked by TRF or Tocomin when fed together with cholesterol rich diet. Similarly, TRF and Tocomin significantly blocked the LDL oxidation and restored the lag phase time close to normal value. The combined results demonstrate that strong hypolipidemic impacts of TRF or Tocomin in conjunction with it’s potent antioxidant property can provide an additional therapeutic benefit in the prevention and treatment of hyperlipidemia and atherosclerosis. Consistent with above results, feeding of the cholesterol rich diet to rabbits for 22.4 weeks was also associated with the formation of fatty streak lesions in the aortas of hyperlipidemic rabbits. The average areas in aortas of these rabbits covered by fatty streak lesions was 21.5%, whereas TRF or Tocomin supplemented groups revealed an average lesion areas of 10.3% and 13.3%,
respectively. Therefore, feeding of dietary TRF or Tocomin together with cholesterol rich diet caused a significant reduction in the formation of aortic fatty streak lesions by 2.1- and 1.6-fold, respectively. These results demonstrate that tocotrienols (TRF or Tocomin) being a potent anticholesterol and antioxidant agents mediated a significant protection against the formation of early atherosclerotic lesions in the aortas induced by cholesterol feeding to rabbits.

Based on strong hypolipidemic, antioxidant and antiatherosclerotic properties of tocotrienols (TRF or Tocomin) fed together with a cholesterol rich diet to rabbits for 22.4 weeks; we have investigated the role of dietary oxidized cholesterol in experimental hyperlipidemia, oxidative modification of LDL and in the acceleration of fatty streak lesions in the aorta of rabbits. In addition, therapeutic role of TRF in the prevention and treatment of experimental hyperlipidemia and atherosclerosis induced by cholesterol oxidation products was investigated. After 10 weeks of feeding to rabbits either a 0.33% cholesterol rich diet or the same diet containing 0.33% cholesterol of which 5% was oxidized, plasma TG, TC including FC and EC, VLDL-C, LDL-C, HDL-C and it’s subfractions, HDL3-C and HDL2-C were substantially increased but to a similar extent. Supplementation of 50 mg% of TRF to these diets significantly blocked the increase in the above lipid parameters, except HDL-C, HDL3-C and HDL2-C levels were not reduced significantly. Similarly, HDL-C/ TC and HDL-C/ LDL-C ratios were increased, whereas TC/ HDL-C and LDL-C/HDL-C ratios were reduced in TRF-treated rabbits, when compared to ratios obtained from respective hyperlipidemic controls, indicating a strong antiatherogenic property of tocotrienols. In contrast to plasma TC, feeding of cholesterol rich diet containing 5% oxidized cholesterol was associated with a significantly higher increase in hepatic TC level than nonoxidized cholesterol fed rabbits. In addition, the decrease in liver TC mediated by TRF in oxidized cholesterol fed rabbits was two-fold greater than TRF plus nonoxidized cholesterol fed animals. The combined results
demonstrate a strong cholesterol lowering property of tocotrienols (TRF), which were significantly more effective in the livers of oxidized cholesterol fed rabbits.

The oxidative stress, evoked in oxidized cholesterol fed rabbits was significantly higher than nonoxidized cholesterol fed rabbits. This differential effect was reflected in several indices of oxidative stress, such as plasma and liver lipid peroxidases, baseline levels of ex vivo diene conjugation of LDL, rates of conjugated diene formation and TBARS contents of LDL, and lag phase time of in vitro LDL oxidation. These oxidative parameters were substantially more pronounced in rabbits fed oxidized cholesterol, apparently due to the presence of cholesterol oxidation products. Tocotrienols being very potent antioxidants, significantly blocked the above mentioned oxidative parameters in both groups. However, tocotrienols were significantly more potent in blocking the increasing plasma and liver lipid peroxidases as well as ex vivo and in vitro LDL oxidation, when supplemented with a diet enriched in oxidized cholesterol. Feeding of 0.33% cholesterol rich diet containing 5% oxidized cholesterol, which is rich in cholesterol oxidation products, caused a further increase in oxidative stress, which in turn decreased the lag time from a control value of 75 to 15 min, in comparison to a decline of 30 min in nonoxidized cholesterol fed rabbits. Similarly, feeding of TRF together with oxidized cholesterol rich diet significantly increased the resistance of LDL to oxidative modification, as shown by an increase in lag time from 15 to 70 min, which is very close to normal value of 75 min. However, in nonoxidized cholesterol plus TRF fed rabbits the restoration of lag time was only from 30 to 60 min.

Consistent with the above results, feeding of nonoxidized cholesterol rich diet resulted in the formation of fatty streak lesions in the aorta of rabbits, which covered an area of 14.3%. An average fatty streak lesion area of 27.7% was found in the aortas of rabbits fed a cholesterol rich diet containing 5% oxidized...
cholesterol. Thus, intake of a very small quantity, that is, 16.4 mg/day of dietary oxidized cholesterol increased the fatty streak lesions by \( \sim 100\% \). These results demonstrate that cholesterol in the diet is considerably more atherogenic when part of it is in the oxidized form. Consistent with a strong hypolipidemic and antioxidant action of tocotrienols, supplementation of TRF with oxidized cholesterol blocked the formation of fatty streak lesions by 4.1-fold, whereas TRF feeding together with nonoxidized cholesterol caused a reduction of 2.4-fold. These results show that the efficacy of dietary tocotrienols in terms of inhibiting the formation of vascular lesions in oxidized cholesterol fed rabbits was significantly higher than non-oxidized cholesterol plus TRF fed animals. These results indicate that for moderate experimental hypercholesterolemia, a situation more relevant to physiological hypercholesterolemia in humans, circulating cholesterol oxidation products may play an important role in inducing formation of early atherosclerotic lesions. Because cholesterol oxidation products are often present in cholesterol containing diets, foam cell lesion formation induced by oxidized cholesterol rather than cholesterol needs to be further investigated. In addition, the mechanism of action of tocotrienols in the inhibition of accelerated aortic atherosclerosis in rabbits fed a cholesterol rich diet containing 5% oxidized cholesterol also needs further investigation.

It is well established that Western as well as Asian, including Indian diets, contain high concentrations of oxidized cholesterol products, and our results suggest that these foods may be a risk factor for atherosclerosis. Based on our combined results of long-term (22.4 weeks) cholesterol feeding experiment and a 10-week cholesterol feeding experiment containing 5% oxidized cholesterol, it can be concluded that dietary tocotrienols (TRF or Tocomin) are potent hypolipidemic, antioxidant and antiatherosclerotic agents. Therefore, daily intake of dietary TRF or Tocomin will be useful in the prevention and treatment of
atherosclerosis. Furthermore, tocotrienols (TRF or Tocomin) will be an excellent source of vitamin E with substantial and potent antioxidant activity.