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Protective Role of Dietary Tocotrienols and Black Caraway Oil on Infection and Inflammation Induced Lipoprotein Oxidation in Animals

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ABSTRACT

Coronary heart disease (CHD) is the main cause of disability and premature death worldwide. Angina and Heart attacks are the two most common aspects of CHD and it occurs due to the high cholesterol level. High blood cholesterol results in Atherosclerosis, which is characterized by presence of atheromas. Epidemiological studies have suggested a link between atherosclerosis and inflammation. In this study, we investigated the efficacy of antioxidant and hypolipidemic agents Tocotrienols and Black Caraway Oil (BCO) by analyzing all the parameters in plasma lipoprotein lipids, Total lipids, TC, TG, VLDL-C, LDL-C, non-HDL-C, MDA, and in-vitro oxidizability of LDL as well as hepatic antioxidant enzymes (Catalase, Superoxide dismutase, Glutathione peroxidase and Glutathione reductase), as investigated in inflammation induced hyperlipidemic rats. All the plasma lipids parameters and hepatic antioxidant enzymes were significantly increased/decreased in hyperlipidemic control rats. After 4-week administration of Tocotrienols and BCO significantly restore the above altered parameters. In conclusion, Tocotrienols and BCO may be useful in the prevention and treatment of infection and inflammation induced hyperlipidemia, CHD and atherosclerosis.

Keywords: Tocotrienols, BCO, CHD, Atherosclerosis, Inflammation, Hypolipidemic, Hepatic antioxidant enzymes.

INTRODUCTION

Coronary heart disease (CHD) is the main cause of disability and premature death worldwide, and is projected to remain the leading cause of death. An estimated 17.5 million people died

from this disease in 2005, representing 30 % of all global deaths. Of these deaths, 7.6 million were due to CHD and 5.7 million because of stroke. If immediate and proper attention is not paid, by 2015 an estimated 20 million people will die from Cardiovascular Disease (CVD), including stroke [1]. It is a major public-health challenge, especially in low and middle income countries, where 80 % of these deaths occur. It has been projected that by the year 2010, 60% of the world's patients with heart disease will be in India [2]. Epidemiological studies have suggested a link between atherosclerosis and infection and inflammation. Atherosclerosis is a multifaceted disease process with several different well defined risks factors, such as hypercholesterolemia, smoking, hypertension and diabetes. Infection and inflammation induce the systemic host response known as acute phase response (APR), and produce many abnormalities that could increase the risk of developing atherosclerosis. In animal models, Infection and inflammation are produced by administration of LPS (acute systemic infection), zymosan (acute noninfectious systemic inflammation), and turpentine or croton oil (acute localized sterile inflammation). Lipid disturbances linked to chronic inflammation will undoubtedly participate in the increased cardiovascular risk associated with hyperlipidemia. Lipid disturbances as hypercholesterolemia and low HDL-C levels are major cardiovascular risk factors. The most typical changes in lipoprotein metabolism during infection and inflammation are hypertriglyceridemia. Hypertriglyceridemia, due to an increase in VLDL, is commonly observed and is due to both enhanced hepatic production of VLDL and decreased triglyceride clearance [3]. The acute phase response (APR) represents the initial line of defense against injury as well as bacterial and parasitic infections. Normal cellular metabolism involves the production of ROS [4], low levels of ROS are vital for proper cell functioning, while excessive *in vivo* generation of these products can adversely affect cell functioning [5]. Oxidative damage to cholesterol component of the low-density lipoprotein (LDL) leads to oxidized LDL by a series of consecutive events. This induces endothelial dysfunction, which promotes inflammation during atherosclerosis. Oxidized LDL acts as a trigger to initiate endothelial inflammation leading to atherosclerosis and vascular thrombosis (heart attack and stroke). The last two decades witnessed an enormous research rush to reveal the pharmacological actions of an annual spicy delicate and beautiful herb known by the Latin name *Nigella sativa* that belongs to the botanical family *Ranunculaceae*. Its English name is Black Cumin or Black Caraway. *Nigella sativa* have been used for many biological studies such as protective effect of *Nigella sativa* seed against carbon tetrachloride-induced liver damage. Effects of *Nigella sativa* on oxidative stress and beta-cell damage in streptozotocin-induced diabetic rats [6]. The antioxidative and antihistaminic effect of *Nigella sativa* and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage [6], Effects of *Nigella unguicularis* fixed oil on blood biochemistry and oxidant/antioxidant balance in rats [7]. In this study, we investigate the efficacy of antioxidant, anti-atherogenic and hypolipidemic agents Tocotrienols and BCO by analyzing all the parameters in plasma, TC, VLDL-C, LDL-C, HDL-C (HDL_2-C, HDL_3-C), TBAR, MDA, Hepatic TG, TC and antioxidant enzymes (CAT, SOD, GPx and Gred) as well as *in-vitro* oxidizability of Low Density Lipoprotein.

MATERIALS AND METHODS

Chemicals: 1-Chloro 2, 4-Dinitrobenzene was purchased from Central drug house, Pvt. Ltd. (India). All other chemicals used for this study were of analytical grade and obtained from HIMEDIA (India), Sisco (India), Ashirwad (India), Sigma-Aldrich (USA), Miles (USA), Acros

(USA) and Tocotrienols drug as well as RBD palm olein were supplied as a gift from CAROTECH BHD, Chemor, Malaysia.

Estimation: Plasma triglyceride [8], Plasma Cholesterol, LDL and HDL [9], Plasma VLDL-C [10], Fractionation of Plasma lipoprotein such as LDL [11], HDL and its fractions-HDL_2, HDL_3 [12], Plasma FRAP [13], *ex vivo* and *in vitro* Cu^{++}-mediated LDL oxidation [14, 15] were measured by following known procedures.

Experimental Design: The experimental study was approved by the Dolphin Institute of Biomedical and Natural Science, Dehradun, Uttarakhand, where the study was conducted. The rats were given pelleted rat chow. Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. For the present study, animals were divided into following 4 groups: Normal control rats (NC); six rats were given 1.0 ml saline/rat/day through gastric intubation for 4 weeks, Inflammation induced Hyperlipidemic control rats (IIHC); six rats were given 1.0 ml saline/rat/day through gastric intubation for 4 weeks, Inflammation Induced Hyperlipidemic Tocomin Treated Rats (IIH-T_3); six rats were given 6.0 mg Tocotrienols/rat/day through gastric intubation for 4 weeks and Black Caraway Oil Treated Rats (BCO-T); six rats in this group were given 1.0 ml Black Caraway Oil/rat/day through gastric intubation for 4 weeks.

Induction of Inflammation:
Inflammation was induced in IIH-C and BCO-T by the subcutaneous injection of turpentine (0.5ml/rat) in the dorsolumbar region and left for five hours.

Collection of Blood and Plasma: For the estimation of different parameters, overnight fasted rats in each group were anaesthetized and blood drawn from cardiac puncture, and were collected in heparinised tube. Plasma was separated from blood by centrifugation at 2500 rpm for 30 min.

Statistical evaluation: This was done by employing two-tailed Student t-test as described by Bennet and Franklin [16]. P value less than 0.02 were considered significant.

RESULTS

Average Body Weight in each Group of Rats before and after 4 Weeks of Tocotrienols and Black Caraway Oil Treatment: As shown in Table 1, the average body weight (g) of normal control rats (N-C), Inflammation induced hyperlipidemic rat (IIH-C), and Inflammation induced hyperlipidemic Tocotrienols treated rats (IIH-T_3T) and Black Caraway Oil treated (BCO-T) was 168, 167, 175 and 170 (g), whereas, the average body weight of N-C, IIH-C, IIH-T_3 and BCO-T rats showed a significant gain of 34%, 20%, 52% and 24% respectively after 4 weeks of treatment. These results demonstrate that in Inflammation induced hyperlipidemic Tocotrienols and BCO treated rats the gain in body weight after 4 weeks was significantly higher than rats in N-C group.

Group	Average Body Weight (g)	
	Before Treatment	After Treatment
N-C	168.12±2.65*	224.82±15.58 (+33.73%)[a]
IIH-C	167.82±3.89*	202.10±18.09 (+20.43%)[a]
IIH-T_3	175.26±7.84*	267.24±13.12 (+52.48%)[a]
BCO-T	170.25±6.03*	211.52±13.39 (+24.24%)[a]

Table 1: Average body weight in each group of rats before and after 4 weeks of tocotrienols and Black caraway oil treatment. *Values are mean ± SD from 6 rats in each group. N-C, normal control; IIHC Inflammation induced hyperlipidemic rats, IIH-T_3, Inflammation induced hyperlipidemic tocotrienols treated rats fed 6.0 mg Tocotrienol/rat/day and BCO-T, Black Caraway Oil treated, given 1.0 ml Black Caraway/rat/day for 4 weeks. Significantly different from N-C at [a]p<0.001. Significantly different from IIH-C at [a]p<0.001.

Impacts of Tocotrienols and Black Caraway Oil (BCO) on Plasma Lipids, Plasma Lipoprotein Lipids, and Lipid Peroxidation Status in Plasma, Liver in Inflammation induced Hypercholestrolemic Rats Treated for 4 Weeks: In the experiments described below the efficacy of Tocotrienols (6.0 mg/rat/day) and BCO (1.0 ml/rat/day), in preventing the increase in lipid parameters, lipid Peroxidation and oxidative stress was investigated in Inflammation induced hyperlipidemic rats, after 4 weeks of administration.

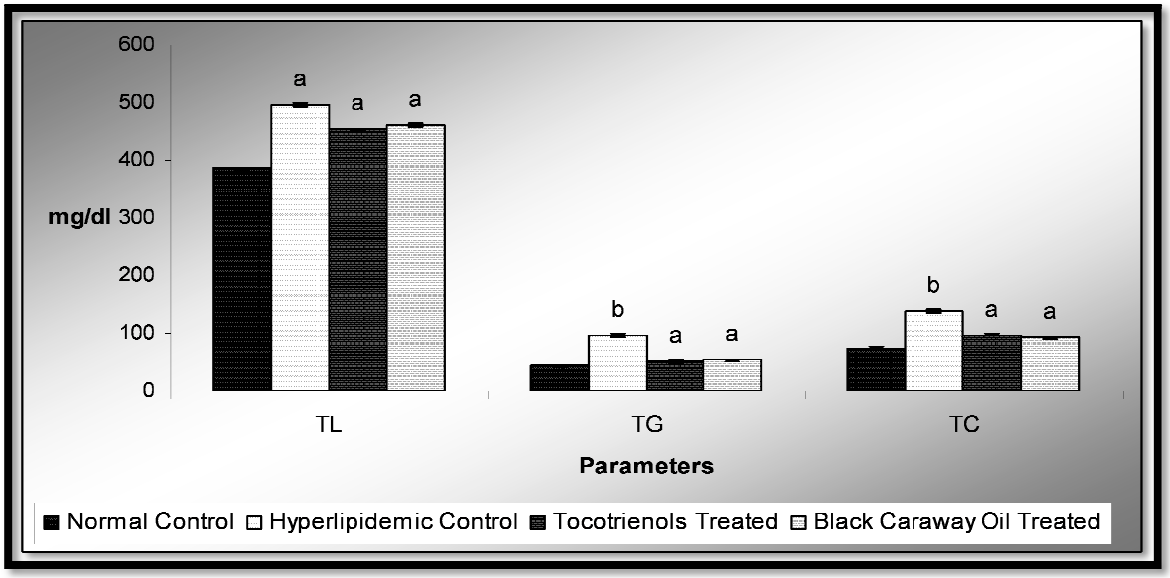

Fig. 1: Impacts of Tocotrienols and BCO on Plasma Total Lipid (TL), Triglycerides (TG) and Total Cholesterol in IIH-C rats after 4 weeks of treatment. *Values are mean (mg/dl) ± SD from pooled plasma of 6 rats in each group. N-C, normal control; IIH-C Inflammation Induced hyperlipidemic rats; IIH-T_3,Inflammation induced hyperlipidemic tocotrienols treated rats fed 6.0 mg Tocotrienols/rat/day and BCO treated rats, given 1.0 ml Black caraway oil/rat/day for 4 weeks. Significantly different from N-C at [a]p<0.001. Significantly different from IIH-C at [a]p<0.001 and [b]p<0.05.

Effects on plasma lipids: As seen in **Fig. 1**, all the plasma lipids parameters were significantly increased in Inflammation induced hyperlipidemic rats (IIH-C) rats, when compared to N-C values. Total lipids (TL), triglycerides (TG) and total cholesterol (TC) significantly increased from 386, 44, and 75 mg/dl in N-C to 495, 97, and 137 mg/dl, respectively, in IIH-C group. After 4 weeks of Tocotrienols treatment, levels of TL, TG, and TC were significantly decreased by 8.5%, 46%, and 29%, respectively, when compared to corresponding IIH-C values. Whereas in BCO treated rats, TL, TG and TC levels were significantly reduced by 7%, 44%, 32% respectively. These results demonstrate that 4-week treatment of IIH-C rats with 6.0 mg Tocotrienols and 1.0 ml BCO mediated a similar and significant reduction in above lipid parameters.

Effects on plasma lipoprotein lipids: **Fig. 2** depicts that, plasma VLDL-C, LDL-C and non-HDL-cholesterol (non-HDL-C) levels were significantly increased from 9, 48 and 50 mg/dl in N-C to 18 mg/dl (105%), 98 mg/dl (104%) and 110 mg/dl (118%) respectively in IIH-C. After 4 weeks of Tocotrienols and BCO treatment, both VLDL-C, LDL-C and non-HDL-C levels showed a significant reduction 40 %, 42 % and 38 % in IIH-T_3, Whereas, in BCO treated rats, VLDL-C, LDL-C and non-HDL-C were significantly reduced of 44%, 46% and 44%, respectively, in comparison to corresponding values in IIH-C rats. HDL-C, HDL_2-C and HDL_3-C levels were decreased from 17, 5 and 11 mg/dl in N-C to 15 mg/dl (12%), 3 mg/dl (40%) and 11 mg/dl (4%), respectively, in IIH-C values.

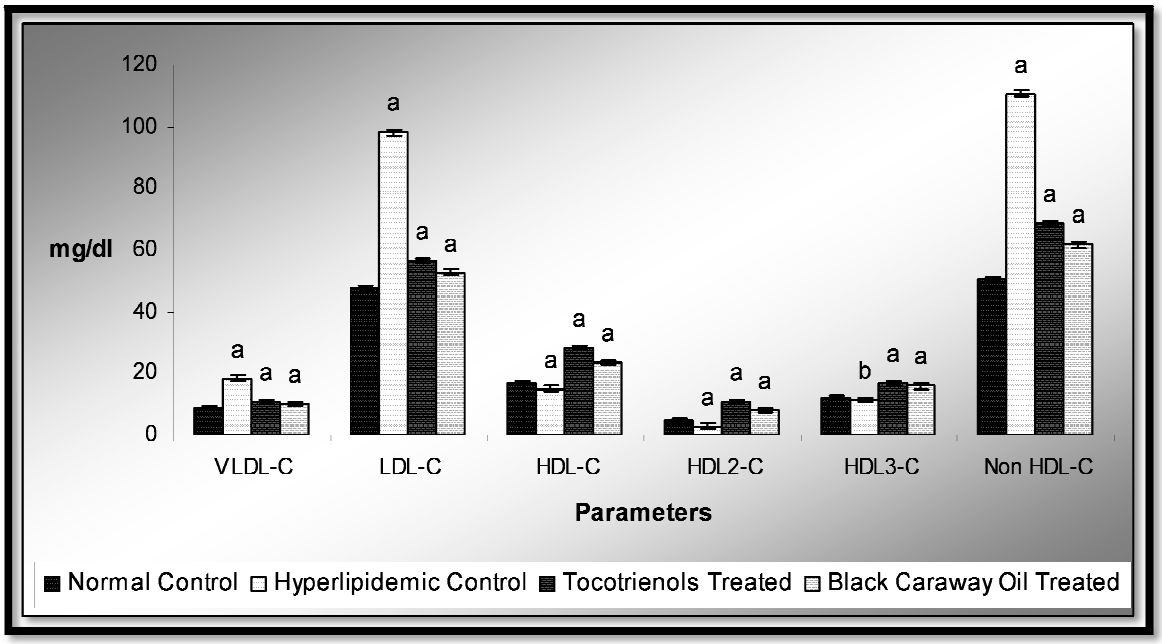

Fig. 2 Impacts of Tocotrienols and BCO on plasma VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C and Non-HDL-C in inflammation induced hyperlipidemic rats after 4 weeks of treatment. *Values are mean (mg/dl) ± SD from pooled plasma of 6 rats in each group. N-C, normal control; IIHC Inflammation Induced Hyperlipidemic rats; IIH-T_3, fed 6.0 mg Tocotrienols/rat/day and Black Caraway Oil treated rats, given 1.0 ml Black Caraway oil/rat/day for 4 weeks. Significantly different from N-C at $^{a}p<0.001$ and $^{c}p<0.02$. Significantly different from IIH-C at $^{a}p<0.001$.

After 4 weeks of Tocotrienols treatment (IIH-T_3) HDL-C, HDL_2-C and HDL_3-C levels showed a significant increase of 86%, 265% and 48%, respectively, when compared to corresponding values in IIH-C, Whereas , in BCO treated rats, HDL-C, HDL_2-C and HDL_3-C levels were increased by 58%, 166% and 39% respectively. These results demonstrate that Tocotrienols and BCO are effective in reducing VLDL-C and LDL-C levels. On the other hand, in comparison to IIH-C values, treatment of IIH-T_3 and BCO mediated a significantly higher increase in HDL-C, HDL_2-C and HDL_3-C concentration.

***Impacts on the ratios of LDL-C/HDL-C and HDL-C/TC*:** As shown in **Table 2**, LDL-C/HDL-C and HDL-C/TC ratios were calculated from the data presented in Fig. 1 and 2. LDL-C/HDL-C ratio was significantly increased from 2.81 in N-C to 6.54 (132 %) in IIH-C group, when compared to ratio in N-C. After 4 weeks of treatment, the increase in LDL-C/HDL-C ratio was significantly prevented and decreased to 2.01 in IIH-T_3, and 2.21 in BCO respectively, which is close to normal control value. On the other hand, HDL-C/TC ratio was significantly decreased from 0.225 in N-C to 0.108 (52 %) in IIH-C group. Tocotrienols and BCO treatment to these rats significantly prevented the increase in HDL-C/TC ratios and fully restored them to a ratio value similar to N-C. In addition, the ratios related to HDL-C in Tocotrienols and BCO treated rats were positively modulated and restored similar to normal control value, indicating normalization of cholesterol levels associated with the above lipoproteins.

Ratio[†]	Group			
	N-C	IIH-C	IIH-T3	BCO-T
LDL-C/HDL-C	2.81±0.022[*]	6.54±0.037[*] (+132.74 %)[a]	2.01±0.027[*] (-69.26 %)[a]	2.21±0.023[*] (-66.20 %)[a]
HDL-C/TC	0.225±0.034	0.108±0.003 (-52.00 %)[a]	0.286±0.079 (+164.81 %)[a]	0.256±0.037 (+137.03 %)[a]

Table 2: Impacts of Tocotrienols and Black Caraway Oil on the ratio of LDL-C/HDL-C and HDL-C/TC after 4 weeks of treatment, [†]For the calculation of ratios, data is taken from Fig. 1 and 2. [*]Values are mean ± SD from pooled plasma of 6 rats in each group. N-C, normal control; IIH-C Inflammation Induced Hyperlipidemic rats; IIH-T_3, fed 6.0 mg Tocotrienols/ rat/ day and BCO-T, given 1.0 ml rat/day for 4 weeks. Significantly different from N-C at [a]$p<0.001$. Significantly different from IIH-C at [a]$p<0.00$.

Impact on plasma total antioxidants and lipid peroxidation products: As seen in Table 6, depicts the antioxidant impact of Tocotrienols and BCO on plasma concentrations of total antioxidants, conjugated diene, lipid hydroperoxide and MDA in Inflammation induced hyperlipidemic (IIH-C) rats. In IIH-C rats, plasma total antioxidants level was reduced from a control value of 50 to 37 (25%) µmole/dl. Treatment of IIH-C rats with Tocotrienols and BCO for 4 weeks resulted in a significant increase of total antioxidants levels by 25% and 14% respectively, when compared to IIH-C value. The oxidative stress induced in IIH-C rats significantly enhanced plasma lipid peroxidation products, such as conjugated diene, lipid hydroperoxide and MDA. Formation of conjugated diene, lipid hydroperoxide and MDA in plasma was increased from 8.57, 1.12 and 1.38 in N-C to 13.09 (52 %), 1.87 (67 %) and 3.12 (126 %) µmole/dl, respectively, in IIH-C. After Tocotrienols treatment, in IIH-T_3, a significant decrease of 19 %, 32 % and 37 % was seen in the formation of conjugated diene, lipid hydroperoxide and MDA, respectively, when compared to corresponding values in IIH-C rats.

Similarly in BCO-T group, conjugated diene, lipid hydroperoxide and MDA in plasma were also significantly decreased by 11%, 29%, 35% respectively, when compared to corresponding values in IIH-C rats. These results demonstrate that in IIH-C rats, due to increase in oxidative stress, total antioxidants level was decreased, whereas, concentration of plasma conjugated diene, lipid hydroperoxide and MDA were significantly increased. Tocotrienols and BCO treatment significantly restored the total antioxidants level and blocked the increase in plasma conjugated diene, lipid hydroperoxide and MDA to a level close to corresponding normal values.

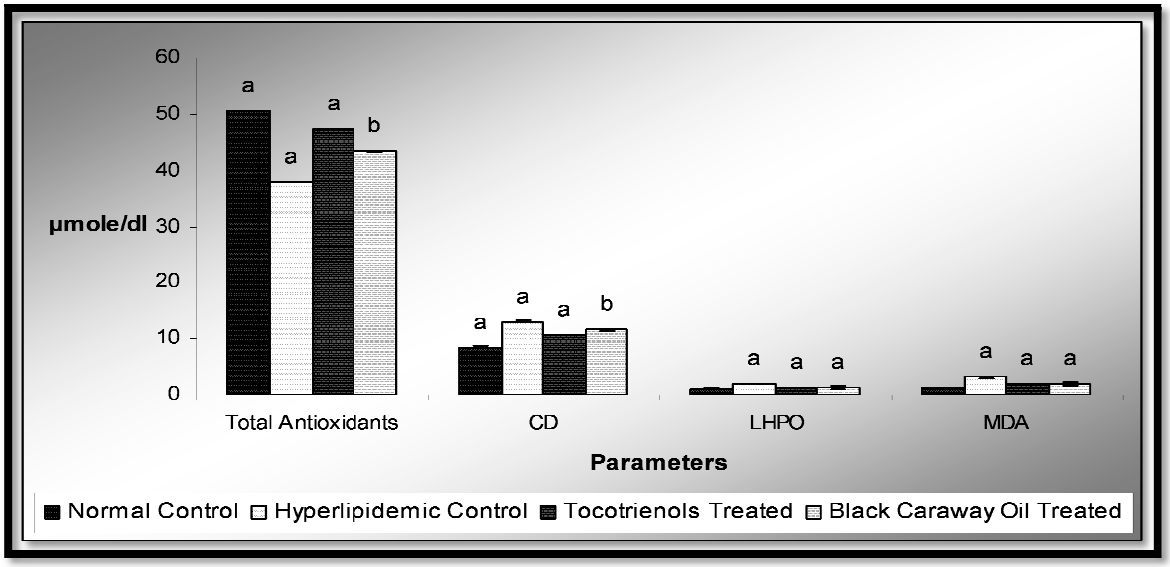

Fig.3: Impacts of Tocotrienols and Black Caraway Oil on plasma Total Antioxidants, Conjugated diene (CD), Lipid hydroperoxide (LHPO) and Malondialdehyde (MDA) contents in IIH-C rats after 4 weeks of treatment. *Values are mean (µmole/dl) ± SD from pooled plasma of 6 rats in each group. N-C, normal control; IIHC Inflammation Induced Hyperlipidemic rats; IIH-T_3, fed 6.0 mg Tocotrienols/ rat/ day and BCO-T, given 1.0 ml /rat/day for 4 weeks. Significantly different from N-C at $^{a}p<0.001$. Significantly different from IIH-C at $^{a}p<0.001$ and $^{b}p<0.05$.

***Lipid Lowering Effect on liver triglycerides and total cholesterol*:** As seen in **Fig. 4**, hepatic levels of triglyceride (TG) and total cholesterol (TC) were significantly increased in Inflammation induced hyperlipidemic rats (IIH-C) by 34% and 137% respectively, when compared to corresponding values in N-C. Feeding of Tocotrienols and BCO to IIH-C rats for 4 weeks was associated with a significant decline in liver TG and TC levels by 14% and 38% respectively, in IIH-T_3, Whereas, in BCO-T group, TG, TC levels were reduced by 6%, 41% respectively, when compared to corresponding value in IIH-C. These results demonstrate that similar to plasma TG and TC level in liver was significantly increased in IIH-C rats. In addition, feeding of Tocotrienols and BCO to IIH-C rats resulted in a significant decline of TG and TC to a level similar to corresponding values in N-C. The combined results demonstrate that levels of TG, TC in plasma and liver lipids were significantly increased in IIH-C rats. Treatment of these stressed rats with 6.0 mg Tocotrienols/rat/day and 1.0 ml BCO/rat/day mediated a significantly decline in the above lipid parameters, similar to corresponding values in N-C rats.

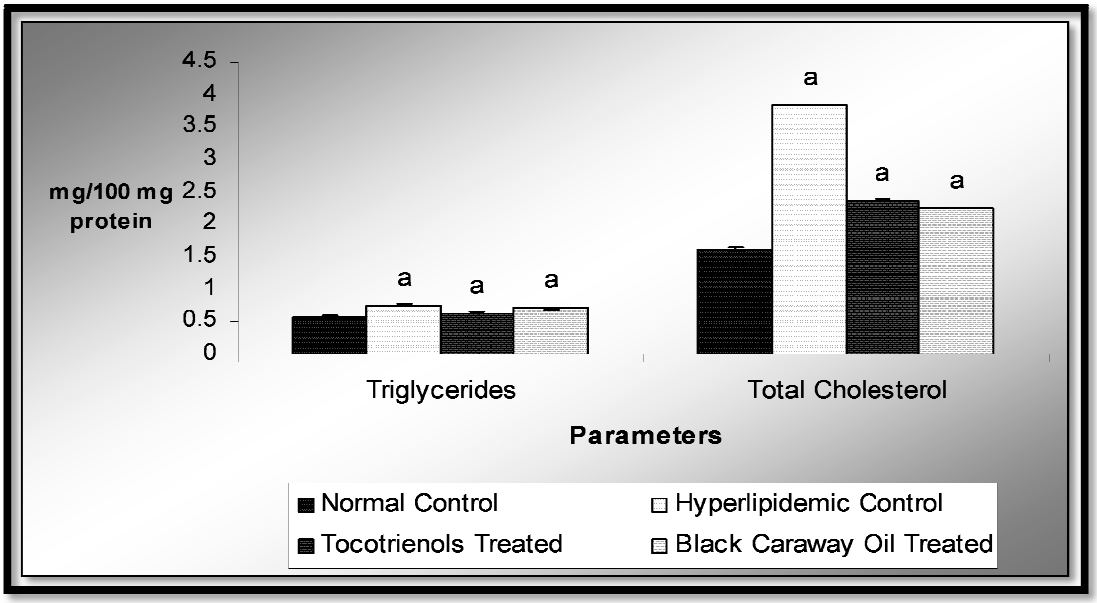

Fig. 4: Impacts of Tocotrienols and Black Caraway Oil on Liver Triglycerides and Total Cholesterol contents in IIH-C rats after 4 weeks of treatment. [*]Values are mean ± SD from homogenate of pooled liver, of 6 rats in each group. N-C, normal control; IIHC; IIH-T_3, fed 6.0 mg Tocotrienols/rat/day and BCO-T, given 1.0 ml /rat/day for 4 weeks. Significantly different from N-C at [a]p<0.001. Significantly different from IIH-C at [b]p<0.05

***Impacts on liver lipid peroxidation products*:** As seen in Fig. 6, the formation of conjugated diene, lipid hydroperoxide and MDA in liver of Inflammation induced hyperlipidemic (IIH-C) rats were significantly increased by 55%, 34% and 54%, respectively. Feeding of Tocotrienols and BCO to hyperlipidemic rats for 4 weeks, was associated with a significant decline in the formation of liver conjugated diene, lipid hydroperoxide and MDA by 30%, 21% and 45% in IIH-T_3 rats, whereas, in BCO-T, these levels were reduced by 28%, 19%, 25% respectively when compared to corresponding values in IIH-C group. These results demonstrate that increased levels of conjugated diene, lipid hydroperoxide and MDA in liver of IIH-C rats were significantly reduced after 4 weeks of Tocotrienols and BCO treatment.

Antioxidant effect on basal levels of conjugated diene formation and lag phase in LDL: As depicted in **Table 3**, the *ex vivo* base line diene conjugation (BDC) levels of LDL in Inflammation induced hyperlipidemic (IIH-C) rats was increased by 65% respectively, in comparison to the corresponding N-C values. Feeding of Tocotrienols to IIH-C rats partially blocked the *in vivo* oxidation of LDL and reduced their BDC levels by 26% respectively. Similarly, after BCO treatment, BDC level in was reduced by 19% respectively in comparison to the corresponding IIH-C values. As expected, the lag phase time of LDL oxidation was reduced from 89 min in N-C to 54 min in IIH-C. Treatment of hyperlipidemic rats with Tocotrienols and BCO restored the lag phase time of LDL oxidation to 74 and 64 min respectively.

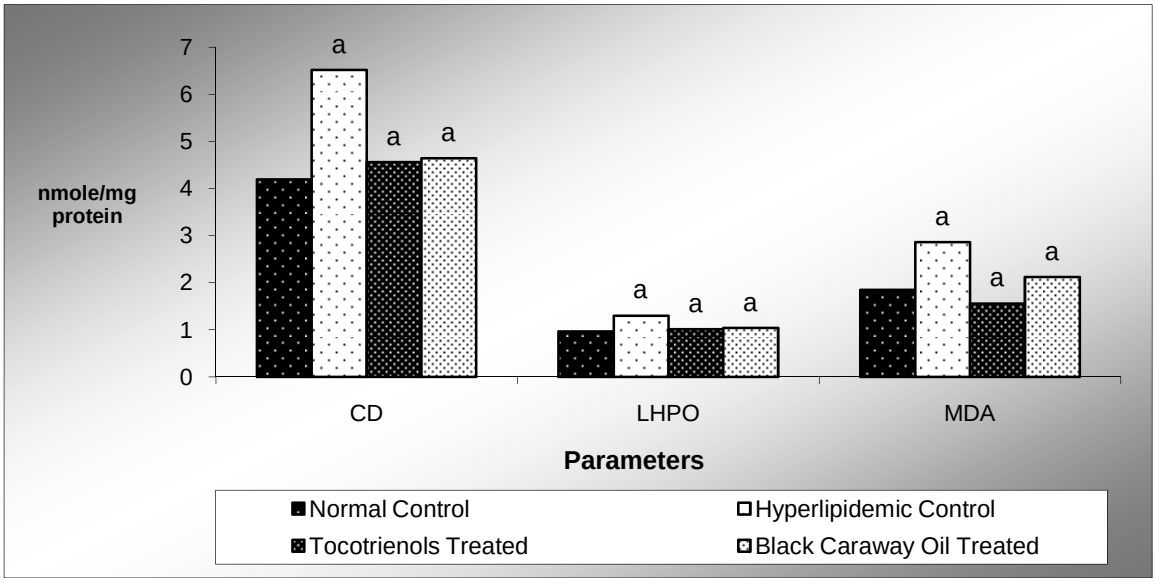

Fig. 6 Impacts of Tocotrienols and Black Caraway Oil on liver lipid peroxidation products (Conjugated diene, Lipid hydroperoxide and Malondialdehyde) after 4 weeks of treatment. [*]Values are mean (nmole/mg protein)± SD from homogenate of pooled liver of 6 rats in each group. N-C, normal control; IIH-C IIH-T_3, fed 6.0 mg Tocotrienol/rat/day and BCO-T, given 1.0 ml /rat/day for 4 weeks. Significantly different from N-C at [a]p<0.001. Significantly different from IIH-C at [a]p<0.001.

Group	LDL Oxidation	
	Basal[*]	Lag Phase[$]
N-C	166.45[+]	89
IIH-C	275.32[+] (+65.40%)[†]	54 (-39.32%)[¶]
IIH-T_3	202.54[+] (-26.43%)[††]	74 (+37.03%)[§]
BCO-T	221.49[+] (-19.55%)[††]	64 (+14.64%)[§]

Table 3: Ex vivo and Copper mediated in vitro oxidation of LDL in IIH-C rats after 4 weeks of Tocotrienols and Black Caraway Oil treatment. [*]The conjugated diene values are expressed as nmole malondialdehyde equivalents/mg protein. Basal conjugated diene represent the status of oxidized LDL, *in vivo*. [$]The lag phase is defined as the interval between the intercept of the tangent of the slope of the curve with the time expressed in minutes. [+]Values are obtained from LDL isolated from pooled plasma of 6 rats in each group. N-C, normal control; IIHC; IIH-T_3, fed 6.0 mg Tocotrienols/rat/day and BCO-T, given 1.0 ml /rat/day for 4 weeks. [††]Percent increase with respect to basal value in N-C. [††]Percent decrease with respect to basal value in IIHC. [¶]Percent decreases with respect to lag phase value in N-C. [§]Percent increase with respect to lag phase value in IIH-C.

Impacts of Tocotrienols and Black Caraway Oil (BCO) on the regulation of antioxidant enzymes activities after 4 weeks of treatment*:* As seen in Table 4, Catalase activity in liver was significantly decreased from a value of 3.65 units in N-C to 2.29 (37 %) in Inflammation induced hyperlipidemic rats (IIH-C), respectively. Administration of Tocotrienols to IIH-C

resulted in a significant increase in liver catalase activities by 3.18 (38 %) unit, respectively. In BCO treated group, liver catalase activity was significantly increased by 34%. However, in comparison to corresponding tissue values of normal control rats (N-C), the decline in hepatic SOD activity of IIH-C rats was 27%. Treatment of Tocotrienols and BCO to IIH-C rats resulted in a significant increase in hepatic SOD activity by 24 % and 22%, respectively from normal value.

Group	Liver homogenate	
	Catalase[†]	Superoxide dismutase[‡]
N-C	3.65±0.135[*]	0.751±0.002
IIH-C	2.29±0.216[*] (-37.26 %)[a]	0.547±0.002 (-27.16 %)[a]
IIH-T3	3.18±0.115[*] (+38.86 %)[a]	0.682±0.003 (+24.68 %)[a]
BCO-T	3.08±0.022[*] (+34.49 %)[a]	0.668±0.005 (+22.12 %)[a]

Table 4: Impacts of Tocotrienols and Black Caraway Oil on liver Catalase (CAT) and Superoxide dismutase (SOD) activities in IIH-C rats after 4 weeks of treatment. [†]One unit (U/mg protein) of enzyme activity is defined as the µmoles of H_2O_2 decomposed/min/mg protein. [‡]One unit (U/mg protein) of enzyme activity is defined as the amount of enzyme required to inhibit O.D. at 560 nm of chromogen production by 50 % in one minute. [*]Values are mean ± SD from PMS fraction of pooled liver, of 6 rats in each group. N-C, normal control; IIH-C ; IIH-T_3, fed 6.0 mg Tocotrienols/rat/day and BCO-T, given 1.0 ml /rat/day for 4 weeks. Significantly different from N-C at [a]$p<0.001$ and [b]$p<0.01$. Significantly different from IIH-C at [a]$p<0.001$ and [b]$p<0.05$.

Table 5 summarizes, the results of Gpx and Gred activities in liver of Inflammation induced hyperlipidemic rats (IIH-C) after 4 weeks of Tocotrienols and BCO treatment. In IIH-C rats, Gpx activity in liver were significantly increased from a value of 54 units in N-C to 68 (25 %), units, respectively, in IIH-C rats. As evident, after 4 weeks of treatment with Tocotrienols, Gpx activity in liver was significantly decreased by 29 % .In BCO-T group, the Gpx activity in liver was decreased by 29% respectively, when compared to corresponding tissues values in IIH-C group. On the other hand, in IIH-C rats, the enzymatic activities of hepatic Gred was decreased significantly by 35 % respectively, when compared to corresponding values of N-C rats. Feeding of Tocotrienols and BCO to IIH-C rats significantly blocked the decrease in hepatic Gred activities and increased it to a similar value of 32% and 35%, respectively.

Administration of Tocotrienols and BCO to IIH-C rats significantly prevented the decrease in Gred activity and increased to a level, which is similar to normal value, In summary, hepatic catalase, SOD, Gpx and Gred enzymes, which constitute a mutually supportive team of defense against free radicals (ROS), are significantly decreased in IIH-C rats. However, feeding of Tocotrienols and BCO substantially quenches these free radicals, thus positively normalizing the above enzyme levels.

Group	Liver homogenate	
	Glutathione peroxidase[†]	Glutathione reductase[‡]
N-C	54.49±1.04[*]	8.57±0.209
IIH-C	68.58±1.48[*] (+25.85%)[a]	5.52±0.263 (-35.58%)[a]
IIH-T_3	48.26±1.19[*] (-29.62%)[a]	7.31±0.132 (+32.42%)[a]
BCO-T	48.46±1.86[*] (-29.33%)[a]	7.45±0.213 (+34.96%)[a]

Table 5: Tocotrienols and Black Caraway Oil mediated effects on Liver Glutathione Peroxidase (Gpx) and Glutathione reductase (Gred) activities in IIH-C rats after 4 weeks of treatment. [†]One unit (U/ mg protein) of enzyme activity is defined as nmole oxidized glutathione formed/min/mg homogenate protein. [‡]One unit (U/ mg protein) of enzyme activity is defined as nmole NADPH oxidized/min/mg PMS protein. [#]One unit (U/ mg protein) of enzyme activity is defined as the nmole of 1-chloro 2,4-dinitrobenzene (CDNB) conjugate formed/min/mg PMS protein Values are mean ± SD from homogenate or PMS fraction of pooled liver of 6 rats in each group. N-C, normal control; IIH-C; IIH-T_3, fed 6.0 mg Tocotrienol/rat/day and BCO-T, given 1.0 ml/rat/day for 4 weeks. Significantly different from N-C at [a]$p<0.001$. Significantly different from IIH-C at [a]$p<0.001$.

DISCUSSION

The present study demonstrates the extensive proatherogenic changes, that occurred as a part of the host response to turpentine (acute localized sterile inflammation) administration, on a variety of parameters, like, plasma and lipoprotein lipids in plasma, liver lipid peroxidation products, *ex vivo* and *in vitro* oxidizability of LDL, erythrocytes MDA release; erythrocytes, liver and plasma total antioxidant. Pretreatment of stressed rats with Tocotrienols and Black Caraway Oil (BCO) significantly reduced the overall oxidative burden and effectively ameliorated the above altered parameters, thus, indicating a potent atheroprotective effect of Tocotrienols and BCO. Several epidemiological studies suggest a link between infection/inflammation and atherosclerosis. During the acute-phase response (APR) to infection and inflammation, cytokines induce tissue and plasma events that lead to a wide variety of changes in the plasma concentrations of lipids and lipoproteins. Because these changes in lipids and lipoproteins are similar to those proposed to promote atherogenesis, they may initiate or aggravate atherosclerosis if the course of infection or inflammation is prolonged [3]. Atherosclerosis is a complex process, and atherosclerotic lesions in the arterial wall are characterized by lipid accumulation in macrophages, resulting in foam cell formation [17]. The development of lipid-filled foam cells is primarily regulated by two major determinants: lipid uptake and lipid removal. In epidemiological studies, several risk factors for atherosclerosis have been identified. Hypercholesterolemia, especially elevated levels of LDL-C, is one major risk factor for Coronary Artery Disease (CAD), and therapeutic reductions of TC and LDL-C levels result in a decrease in cardiovascular morbidity and mortality [18]. Hypertriglyceridemia and elevated levels of TG rich VLDL have recently reemerged as risk factors for atherosclerosis [19, 20]. On the other hand, plasma HDL-C levels are inversely correlated with the risk of atherosclerosis [21]. In agreement with these concepts, therapies that raise HDL and decrease triglyceride reduce the morbidity and mortality from CAD

[21]. These intervention trials support the concept that LDL and possibly VLDL play a pivotal role in lipid uptake and that HDL is a key lipoprotein in lipid removal from macrophage foam cells in the arterial wall. Our results demonstrate a significant increase in plasma total lipids and TG in turpentine (IIH-C) stressed rats. In another report an increase in plasma TG level was seen during inflammation, induced by turpentine oil in pigs [22]. The increase in plasma TG levels is apparently due to an increase in VLDL which can be the result of either increased VLDL production or decreased VLDL clearance. These results are consistent with the finding that low doses of LPS stimulate VLDL production, whereas high doses of LPS inhibit VLDL clearance in rats. Therefore, tocotrienols may exert their cholesterol lowering effect in inflammation /infection induced hyperlipidemic rats in a similar manner as previously reported for hyperlipidemic animals [23, 24, 25] and humans [26, 27]. Mechanism wise, as previously shown in HepG2 cells, as well as in normolipidemic and hyperlipidemic rats, tocotrienols reduce cholesterol synthesis by suppressing HMG-CoA reductase activity, which in turn is reduced by a decline in its protein mass [23, 28]. The decline in protein mass may be achieved by inhibition of HMG-CoA reductase synthesis and/or enhanced degradation. Consistent with *in vivo* results in rats [23], γ-tocotrienol has been shown to mediate the suppression of enzymatic activity and protein mass of HMG-CoA reductase in HepG2 cells through decreased synthesis (57 % of control) and enhanced degradation (2.4-fold versus control) of the enzyme [28]. In addition, γ-tocotrienol was shown to upregulate LDL receptor in mammalian cells and may be implicated in part for the reduction of apoB-lipoprotein *in vivo* [28]. Thus, tocotrienols reduce cholesterol formation in mammalian cells by suppressing HMG-CoA reductase activity through two actions: decreasing the efficiency of translation of HMG-CoA reductase mRNA and increasing the controlled degradation of HMG-CoA reductase protein, post-transcriptionally [28]. In addition, another report indicates that γ-tocotrienol influences apoB secretion by both co-translational and posttranslational processes involving a decreased rate of apoB translocation and accelerated degradation of apoB in HepG2 cells. This activity correlated with a decrease in free and esterified cholesterol [29]. Taken together, the information indicates an association between the suppression of hepatic cholesterol synthesis and apoB secretion, and the observed lowering of apoB and LDL-C levels in animal and human models [29]. Our data show that systemic oxidation of lipid/lipoprotein particles occurs as a part of the host response to infection and inflammation. Conjugated diene (which measure the initial phase of lipid peroxidation), lipid hydroperoxide (intermediate product of lipid peroxidation) and MDA (which measure the degradation phase of lipid peroxidation) in plasma, erythrocytes and liver are significantly increased in rats after, turpentine administration. A similar increase in serum conjugated diene and MDA has been reported in IIH-C stressed rats. However, protective effect of an antioxidant agent was not investigated. The increase in plasma lipid peroxidation products is associated with a significant decline in total antioxidants capacity of plasma. The former suggests increased production of oxidants while the latter indicates diminished antioxidant defense. Both the changes indicate an existence of profound oxidative stress. These results are in concordant with well known pro-oxidant properties of turpentine. Our results indicate a significant decrease in plasma lipid peroxidation products with a concomitant and significant increase in plasma total antioxidants in IIH-C group, pretreated with 6.0 mg Tocotrienols/day and 1.0 ml BCO/rat/day for 28 days before turpentine injection. Our study suggests that due to constant availability of antioxidants, in the body of treated hamsters, the integrity of erythrocytes membrane is significantly improved as shown by substantial protection against *in vivo* and *in vitro* H_2O_2 induced lipid peroxidation. Oral pretreatment of rats with Tocotrienols and BCO for 28 days

significantly prevented the turpentine induced adverse effects and ameliorated the levels of all the evaluated parameters. Our results strongly suggest that the alleviation of inflammatory conditions is due to potent lipid lowering and free radical scavenging properties of Tocotrienols and, thus, can be useful in the therapy of systemic inflammatory process which might induce atherosclerosis. Based on these findings, the anti-inflammatory potential of Tocotrienols and BCO looks promising and more comprehensive studies should be undertaken to determine their actual mode of action. In conclusion, considering the strong hypolipidemic/atheroprotective and antioxidant, and possibly anti-inflammatory actions of Tocotrienols and BCO, intake of these may be useful in the prevention and treatment of infection/inflammation induced hyperlipidemia and atherosclerosis without any side effects.

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