Role of Borage Seed Oil and Fish Oil with or without Turmeric and Alpha-Tocopherol in Prevention of Cardiovascular Disease and Fatty Liver in Rats

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Abstract: The aim of the present research was to Study the prevention of dyslipidemia, oxidative stress, inflammation and fatty liver as risk factors for cardiovascular disease via intervention by borage oil (B) and fish oil (F) with or without turmeric (T) and alpha-tocopherols (TC). Fatty acids were assessed in both oils while curcuminoids were determined in turmeric. Rats were divided into; first group fed on balanced diet and designated as normal control (NC), second fed on dyslipidemic and steatohepatitis (DS) inducer diet which represented the DS control group and groups 3-6 fed on DS inducer diet with daily oral administration of B, B+T+TC, F and F+T+TC; respectively for 5 weeks. Liver fat and plasma lipid profile, oxidative stress and inflammatory biomarker and liver and heart histopathology were assessed. Results showed gamma linolenic to be 21.01% in B. F contained eicosapentaenoic as 22.768% and docosahexaenoic acid as 13.574%. Total curcuminoids were 4.63 mg/g turmeric. The DS control group showed significant dyslipidemia, elevated malondialdehyde (MDA), tumor necrosis factor-alpha and liver fat with significant reduction in total antioxidant capacity (TAC) compared to NC. The different treatments produced significant improvement in all the parameters and histopathology. F was superior to B in ameliorating liver histopathological changes while B was more efficient in elevating TAC. B was more promising in improving lipid profile and liver fat compared to B + T + TC, while the latter was superior in improving MDA and liver histopathology. Fish oil was more efficient than F+TC+T except for TAC and high density lipoprotein cholesterol which were more improved on addition of TC and T. Conclusion: Borage and fish oil with or without antioxidants protect from cardiovascular and fatty liver diseases with variable degrees.

Key words: borage oil, fish oil, turmeric, alpha-tocopherol, cardiovascular disease, fatty liver

1 Introduction
Cardiovascular diseases (CVDs) are considered as the major cause of morbidity and death worldwide in both developed and developing countries1. Dyslipidemia, oxidative stress and inflammation are among the reported risk factors for cardiovascular diseases2. Recently fatty liver with inflammation (steatohepatitis), which is increasing worldwide, is accused as being major player in induction of cardiovascular diseases3. Generally, the condition including dyslipidemia, inflammation, steatohepatitis, oxidative stress and impaired glucose tolerance is called metabolic syndrome.

Two series of polyunsaturated fatty acids represented by omega3 and omega6 could have different efficiency towards dyslipidemia and CVDs. Omega3 fatty acids like alpha-linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) possess both hypolipidemic and anti-inflammatory activity but there is a confliction in literature concerning their effect on oxidative stress4. Fish oil is rich in long chain polyunsaturated fatty acids (EPA and DHA) and was reported to possess the aforementioned health effects as well as hepatoprotective effect5. On the other hand little is known about the effect of omega6 fatty acid like linoleic acid on metabolic syndrome. Although linoleic was reported to have hypolipidemic effect but claimed to induce some sorts of pro-inflammation. However; this claim was not
supported by recent studies. Linoleic acid was reported by Maruyama et al. to be a potent protector against lipotoxicity induced inflammation. Other omega6 fatty acid like gamma linolenic (GLA) was reported to have anti-inflammatory and antioxidant effect. The ratio of n-6/n-3 polyunsaturated fatty acids is a better index of health benefit; unfavourable ratio was reported to be associated by greater systemic inflammation.

* Borago officinalis* is native to the Mediterranean region, North Africa and Middle East and is known as starflower or borage. In traditional medicine borage is used in gastrointestinal, respiratory, cardiovascular and urinary disorders. It is also used for pre and post-menopausal symptoms. Commercially, the plant is cultivated for borage seed oil which is used for different medical and nutritional benefits. The seed contain 26-38% oil which is the richest source of gamma linolenic acid (GLA) that accounts for 17-28% of the oil.

Aside from being a popular condiment, turmeric has long history in medicinal use in Ayurvedic medicine for wound healing, hepatic diseases, joint pains, gastrointestinal disorder and cancer prevention. Curcuminoids and phenolic compounds as well as the essential oil were reported to be the major bioactive constituents in turmeric and to which the bioactivity of turmeric was ascribed. The health benefits of such constituents are attributed mainly to their antioxidant and anti-inflammatory effect. A meta analysis study showed curcuminoids therapy to reduce triglycerides after addition of a known volume of chloroform. Assessment of the methyl ester was carried out by injecting 2 μL into a Hewlett Packard HP-system 6890 gas chromatograph equipped with FID. HP-5 capillary column (30 m × 0.32 mm i.d.; 0.25 μm film thickness) was used to separate the different methyl esters. The chromatographic analysis conditions were; initial temperature 70°C with a hold for 1 min, then raised to 120°C at a rate of 40°C/min with 2 min hold then the temperature was finally raised to 220°C at a rate of 4°C/min with another 20 min hold. The injector and detector temperatures were 250°C and 280°C, respectively. Identification of the fatty acid methyl esters were carried out by direct comparison of retention times of each of the separated compounds with standards of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

2.2 Animals
Male Sprague Dawley rats weighing 140-150 g. were used in the present study. Animals were obtained from Animal house of National Research Centre, Cairo, Egypt. Rats were kept individually in stainless steel cages; water and food were given ad libitum. Animal experiment was carried out according to the Medical Research Ethics Committee for institutional and national guide for the care and use of laboratory animals, National Research Centre, Cairo, Egypt.

2.3 Methods
2.3.1 Assessment of fatty acids in borage and fish oil
Methyl esters were prepared via trans-esterification of fatty acids using methanol sulfuric acid and chloroform mixture according to Indarti et al. Twenty mg of the oil was weighed into clean, 10 mL screw-cap glass tubes, to which 4 mL fresh solution of a mixture of freshly prepared methanol, concentrated sulfuric acid and chloroform (1.70/3.2 v/v/v) was added. The tubes were covered; trans-esterification was run at 100°C for 30 min. On completion of the reaction, the tubes were brought to room temperature. Then, 3 mL distilled water was added into the mixture and thoroughly mixed by vortex for 1 min. After the formation of two phases, the lower phase containing the fatty methyl esters was transferred to clean glass tube and dried with anhydrous Na2SO4. Fatty acids were analyzed by GC after addition of a known volume of chloroform.
Chromatographic conditions: The elution was carried out with gradient solvent systems with a flow rate of 1.0 mL/min at ambient temperature. The mobile phase consisted of methanol (A), 2% acetic acid (B), and acetonitrile (C). Quantitative levels of curcuminoids were determined using the above solvents programmed linearly from 45 to 65% acetonitrile in B for 0-15 min. The gradient then went from 65 to 45% acetonitrile in B for 15-20 min, with a constant of 5% A. The compounds were quantified using standard curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

2.3.3 Preparation of Diets
A balanced diet containing 12% casein, 10% Sun-flower oil, 68.5% corn starch, 5% cellulose, 3.5% mineral mixture and 1% vitamin mixture was prepared. Metabolic syndrome (Dyslipidemia and steatohepatitis) inducer diet (DS) composed of 12% casein, 20% coconut oil, 62.25% fructose, 3.5%, mineral mixture, 1% vitamin mixture, 1% cholesterol and 0.25% sodium cholate was prepared utilizing a combination of two previous methods\textsuperscript{25, 21} with some modifications.

2.3.4 Preparation of different nutraceuticals
Different mixtures were freshly prepared every three days to be given to rats. Borago oil (810 mg) was emulsified by tween 80 in distilled water (final volume 15 mL). Tween 80 was used as 10% from the oil. The mixture was mixed well by vortex and designated as nutraceutical 1. Borago oil (810 mg) + α-tocopherol (1.08 g) + turmeric (1.6 g) were emulsified by tween 80 and completed to 15 mL by distilled water and mixed by vortex till became homogeneous (Nutraceutical 2). Tween 80 was used as 10%. Fish oil (810 mg) was prepared as in case of nutraceutical 1 where borago oil was exchanged by fish oil and marked as nutraceutical 3. Nutraceutical 4 was prepared as nutraceutical 2 with replacing borago oil by fish oil. A vehicle was prepared containing the same amount of tween 80 in distilled water as in the nutraceuticals to be given to rats of the control groups.

Turmeric and tocopherol were added together because mixing two types of antioxidant were reported to be more beneficial to health than using only one. Also; tocopherol was added as antioxidant while turmeric possesses both antioxidant and anti-inflammatory effect so the prepared nutraceuticals could afford an antioxidant effect due to two antioxidants from two different sources with simultaneous anti-inflammatory activity.

Alpha-Tocopherol was used previously in a dose of 360 mg/kg rat body weight\textsuperscript{21} i.e 54 mg/150 g rat body weight which was applied in the current study. Turmeric was used as 1100 mg/kg rat body weight according to Rajashekhara et al.\textsuperscript{21} i.e 165 mg/150 g rat body weight. About half the dose of turmeric (80 mg/150 rat body weight) was used in the present study for two reasons; first due to its high viscosity that could not be easy to be given to rats by the stomach tube specially when mixed with the other constituents while the second reason is the presence of another antioxidant represented by α-tocopherol in the nutraceuticals. So in the present study the previously mentioned doses were provided through administration of 0.75 mL of the nutraceuticals 2 and 4.

2.3.5 Design of the animal experiment
Thirty six male rats were divided into 6 groups; each of 6 rats. The first was the normal control (NC) group where the rats received a balanced diet throughout the study period (5 weeks), all other remaining groups were fed on DS inducer diet for 5 weeks. One served as a DS control group, whereas the other 4 groups were fed on DS inducer diet along with an oral administration of a daily oral dose of 0.75 mL from nutraceutical 1, 2, 3 and 4, respectively. The NC and the DS control groups were given daily oral dose of 0.75 mL from the vehicle. During the experiment, body weight and food intake were recorded once a week. At the end of the study which extended for 5 weeks, total food intake and body weight gain were calculated. Blood samples were collected from anaesthetized fasted animals. Plasma was separated from heparinized blood by centrifugation for the determination of different lipid parameters represented by total lipids (T.L), total cholesterol (T.Ch), triglycerides (T.G), low density lipoprotein-cholesterol (LDL-Ch) and high density lipoprotein-cholesterol (HDL-Ch) by colorimetric methods\textsuperscript{21, 22}. Plasma malondialdehyde (MDA) was assessed by the colorimetric methods of Satoh\textsuperscript{23} as oxidative stress biomarker. Plasma total antioxidant capacity (TAC) as an antioxidant biomarker was determined using colorimetric method as reported by Koracevic et al.\textsuperscript{30}. Plasma tumor necrosis factor-α (TNF-α) was estimated as anti-inflammatory biomarker adopting enzyme-linked immunosorbent assay (ELISA) according to the method of Stepaniak et al.\textsuperscript{31}. T.Ch/HDL-Ch was calculated as indicator of cardiovascular disease risk. Rats were dissected and the livers and hearts were separated. A part from each rat liver was kept in deep freeze till analyzed for total lipids according to Cequier-Sánchez et al.\textsuperscript{32} by extraction and gravimetric measure. The other part of each liver together with the heart was preserved in 10% formalin for the histopathology examination.

The different analyzed plasma biochemical parameters were assessed using commercial kits. The reagents kits for T.Ch, T.G and HLD-Ch were obtained from Salucea, Netherlands. Reagents for LDL-Ch were purchased from Centronic GmbH, Germany. T.L, TAC and MDA reagents kits were supplemented from Biodiagnostic, Egypt. Rat TNF-α ELISA kit was obtained from KOMABIOTEH, Seoul, Korea.

2.4 Statistical analysis
The results of animal experiments were expressed as the mean ± SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Tukey
test. In all cases $p < 0.05$ was used as the criterion of statistical significance.

3 Results

3.1 Fatty acid composition of borage and fish oil

The fatty acid composition of borage and fish oil is present in Table 1. It could be noticed that linoleic acid was the major fatty acid (36.482%) followed by $\gamma$-Linolenic (21.01%) in borage oil. Oleic acid was the only monounsaturated acid detected (20.163%). Alpha linolenic acid was present in 2.493%, respectively. The major saturated fatty acid was palmitic followed by stearic and arachidic (10.603, 4.689 and 2.493, respectively). The predominant fatty acid in fish oil was eicosapentaenoic acid (22.788%) and decosahexanoic acid (15.75%)%. The rest of the unsaturated identified fatty acids in fish oil were oleic, palmitoleic, $\gamma$-Linolenic, and linoleic (12.771, 9.349, 3.368 and 2.379%, respectively) while saturated fatty acids were palmitic, myristic and stearic (18.474, 7.452 and 5.243%, respectively).

3.2 Curcuminoids content of turmeric

The major curcuminoid compound in turmeric was curcumin which present as 2.55 mg/g followed by demethoxycurcumin (1.13 mg/g). Bisdemethoxycurcumin showed the least content (0.95 mg/g).

3.3 Results of the animal experiment

Table 2 showed that DS control group demonstrated significant increase in plasma total lipids reflected in the significant elevation of total cholesterol, triglycerides, and LDL-Ch compared to NC. Plasma HDL-Ch decreased significantly along with significant increase of T.Ch/HDL-Ch.

### Table 1 Fatty acids’ content of borage and fish oil (as percentage of total fatty acids)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Borage oil</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid, C14:0</td>
<td>–</td>
<td>7.452</td>
</tr>
<tr>
<td>Palmitic, C16:0</td>
<td>10.603</td>
<td>18.474</td>
</tr>
<tr>
<td>Palmitoleic, C16:1</td>
<td>–</td>
<td>9.349</td>
</tr>
<tr>
<td>Stearic acid, C18:0</td>
<td>4.689</td>
<td>5.243</td>
</tr>
<tr>
<td>Oleic, C18:1</td>
<td>20.163</td>
<td>12.771</td>
</tr>
<tr>
<td>Linoleic, C18:2 (o6)</td>
<td>36.482</td>
<td>2.379</td>
</tr>
<tr>
<td>$\gamma$-Linolenic, C18:3 (o6)</td>
<td>21.010</td>
<td>–</td>
</tr>
<tr>
<td>$\alpha$-Linolenic, C18:3 (o3)</td>
<td>4.559</td>
<td>3.368</td>
</tr>
<tr>
<td>Arachidic acid, C20:0</td>
<td>2.493</td>
<td>–</td>
</tr>
<tr>
<td>EPA, C20:5 (o3)</td>
<td>–</td>
<td>22.768</td>
</tr>
<tr>
<td>DHA, C22:6 (o3)</td>
<td>–</td>
<td>13.574</td>
</tr>
<tr>
<td>Total saturated fatty acids</td>
<td>17.785</td>
<td>31.169</td>
</tr>
<tr>
<td>Total unsaturated fatty acids</td>
<td>62.051</td>
<td>64.209</td>
</tr>
<tr>
<td>Total monounsaturated fatty acids</td>
<td>20.163</td>
<td>22.12</td>
</tr>
<tr>
<td>n-6: n-3 fatty acids</td>
<td>12.66</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### Table 2 Biochemical parameters of different experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>Control DS</th>
<th>DS + Borage oil + turmeric + vitamin E</th>
<th>DS + Borage oil + vitamin E</th>
<th>DS + Fish oil + turmeric + vitamin E</th>
<th>DS + Fish oil + vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.L (mg/dl)</td>
<td>280.2 ± 10.329</td>
<td>658.2 ± 17.696</td>
<td>326.3 ± 28.272</td>
<td>350.5 ± 18.45</td>
<td>312.0 ± 24.59</td>
<td>319.8 ± 40.842</td>
</tr>
<tr>
<td>T.Ch (mg/dl)</td>
<td>81.5 ± 2.437</td>
<td>177.8 ± 5.075</td>
<td>82.9 ± 1.452</td>
<td>99.6 ± 5.35</td>
<td>83.3 ± 5.304</td>
<td>115.3 ± 1.067</td>
</tr>
<tr>
<td>T.G (mg/dl)</td>
<td>92.6 ± 0.217</td>
<td>140.2 ± 0.214</td>
<td>107.3 ± 1.323</td>
<td>114.3 ± 0.201</td>
<td>110.1 ± 0.469</td>
<td>105.6 ± 3.51</td>
</tr>
<tr>
<td>HDL-Ch (mg/dl)</td>
<td>46.5 ± 1.349</td>
<td>31.6 ± 1.389</td>
<td>41.8 ± 1.216</td>
<td>40.4 ± 0.585</td>
<td>44.5 ± 1.044</td>
<td>40.4 ± 0.792</td>
</tr>
<tr>
<td>LDL-Ch (mg/dl)</td>
<td>16.5 ± 1.138</td>
<td>118.1 ± 5.669</td>
<td>19.7 ± 1.092</td>
<td>36.4 ± 2.88</td>
<td>17.3 ± 0.98</td>
<td>53.1 ± 1.323</td>
</tr>
<tr>
<td>T.Ch/HDL-Ch</td>
<td>1.758 ± 0.068</td>
<td>5.687 ± 0.344</td>
<td>1.985 ± 0.068</td>
<td>2.473 ± 0.163</td>
<td>1.87 ± 0.112</td>
<td>2.692 ± 0.129</td>
</tr>
<tr>
<td>TAC (mM/L)</td>
<td>6.08 ± 0.178</td>
<td>3.27 ± 0.247</td>
<td>5.57 ± 0.251</td>
<td>6.04 ± 0.184</td>
<td>4.12 ± 0.251</td>
<td>5.71 ± 0.254</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>3.980 ± 0.606</td>
<td>11.505 ± 0.767</td>
<td>4.327 ± 0.824</td>
<td>2.012 ± 0.421</td>
<td>4.783 ± 0.773</td>
<td>4.843 ± 0.143</td>
</tr>
<tr>
<td>TNF-α (Pg/mL)</td>
<td>51.8 ± 1.415</td>
<td>85.3 ± 0.86</td>
<td>64.7 ± 1.506</td>
<td>63.8 ± 0.495</td>
<td>59.0 ± 2.245</td>
<td>58.5 ± 1.011</td>
</tr>
<tr>
<td>Liver % Total fat</td>
<td>4.2 ± 0.616</td>
<td>7.2 ± 0.290</td>
<td>3.8 ± 0.479</td>
<td>6.1 ± 0.135</td>
<td>3.8 ± 0.222</td>
<td>5.3 ± 0.306</td>
</tr>
</tbody>
</table>

Values are means ± SE; where n = 6. DS: Dyslipidemic and steatohepatitis inducer diet, T.L: total lipids, T.Ch: total cholesterol, T.G: Triglycerides, HDL-Ch: High density lipoprotein cholesterol, TAC: Total antioxidant capacity, MDA: Malondialdehyde, TNF-α: Tumor necrosis factor-alpha

In the same row: Similar superscript letters mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$. 1554
and liver percentage of total lipids in the DS control compared to NC. It was noticed that plasma MDA and TNF-alpha were significantly elevated along with significant reduction of total antioxidant capacity in DS control group compared to NC. Administration of any of the studied nutraceuticals produced significant improvement of all plasma parameters and liver lipids with variable degrees. Fish and borage oil were almost equal in improving all biochemical parameters except for TAC which was more efficiently improved by borage oil. Borage oil was more promising in improving lipid profile and liver fat compared to B + T + TC, while the latter was superior in reducing MDA. Fish oil was more efficient in improving T.Ch, HDL-Ch, LDL-Ch, T.Ch/HDL-Ch, and liver fat compared to F + T + TC; while the latter was more efficient in improving TAC. Compared to normal control; plasma T.Ch, HDL-Ch, T.Ch/ HDL-Ch and MDA as well as liver fats in the groups treated by fish and borage oil showed insignificant change. Treatment by fish oil restored the plasma HDL-Ch to normal level as in NC group. When rats were treated by F + T + TC; the plasma level of MDA was normalized. Plasma TAC of borage oil, B + T + TC and F + T + TC treated rats showed insignificant change compared to NC group. Rats treated by B + T + TC showed promising reduction of MDA which was even significantly lower than NC group.

Different nutritional parameters and percentage organ weight to body weight of different groups are illustrated in Table 3. It was noticed that total food intake was significantly reduced in both DS control group and the treated groups compared to NC group without change in body weight gain. There was no significant change in heart/ body weight% among the different groups while liver/ body weight% was increased significantly in DS control group compared to NC. Treatment with either borage oil or F + T + TC produced significant reduction in liver/ body weight% compared to DS control group while the other treated two groups only showed minor reduction. There was no significant change in liver/ body weight% when the different treated groups were compared with each other.

Histopathological examination of livers and hearts of the different groups are shown in Figs. 1 and 2. It could be noticed from Fig. 1 that liver of NC group showed normal liver structure, the hepatic cords radiating from central vein separated by sinusoid (Fig. 1A). Liver of control DS group demonstrated focal area of hepatic necrosis infiltrated with inflammatory cells and most of hepatocytes showing fatty degeneration (Fig. 1B1). Liver of control DS group also showed fatty degeneration with the hepatocytes taking signet ring appearance (Fig. 1B2). Livers of the group treated by borage seed oil contain multi focal areas of fatty degeneration and focal aggregation of inflammatory cells (Fig. 1C1). Liver of another rat from the group that received borage oil showed proliferation of fibers connective tissue in portal area with inflammatory cells infiltration and fatty degeneration of hepatocytes (Fig. 1C2). Liver of all rats treated by B + T + TC showed marked improvement of histopathological picture (Figs. 1D1 and 1D2). Liver from the majority of rats treated by fish oil showed marked improvement in the histopathological changes (Fig. 1E2). Only one rat from those given fish oil demonstrated mild vacuolar degenerative changes while most of cells are intact (Fig. 1E1). Liver of rats received F + T + TC revealed marked improvement in histopathological picture (Figs. 1F1 and 1F2) while only one rat from this group (Fig. 1F3) showed focal area of vacuolar degeneration.

The improvement of liver histopathological changes could be arranged from the best to the least according to the following sequence; borage combined with T and TC> fish oil = fish oil combined with T and TC> borage oil.

Heart histopathology of different groups (Fig. 2) showed heart of control normal rat with normal histological structure (Fig. 2A). Heart of control DS group contains focal areas of regenerative changes with loss of cardiac myofibers striation and congestion of cardiac blood vessels (Fig.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Nutritional parameters, liver/body weight % and heart/body weight % of different experimental groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Groups</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>147° ± 4.96</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>217.5° ± 6.407</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>68.7° ± 10.026</td>
</tr>
<tr>
<td>Total food intake (g)</td>
<td>622.5° ± 18.786</td>
</tr>
<tr>
<td>Liver/body weight %</td>
<td>2.5° ± 0.062</td>
</tr>
<tr>
<td>Heart/body weight %</td>
<td>0.29° ± 0.118</td>
</tr>
</tbody>
</table>

Values are means ± SE; where n = 6.

DS: Dyslipidemic and steatohepatitis inducer diet

In the same row: The similar superscript letters mean insignificant difference, while different letters mean significant difference between groups at p < 0.05.
Heart of another rat from control DS group showed pericardial edema with separation of myofibers and inflammatory cells infiltration (Fig. 2B2). Generally hearts from the group treated by borage oil demonstrated improvement in histopathological changes (Fig. 2C2) while heart from only one rat from such group showed focal area of myocardial edema with inflammatory cells infiltration (Fig. 2C1). Hearts of rats given borage oil combined with T and TC showed marked improvement in the histopathological picture (Fig. 2D2) while heart from only one rat in the same group still showed loss of striation of cardiac muscles (Fig. 2D3). Hearts of fish oil group showed moderate degenerative changes (Fig. 2E2) while heart from only one rat from such group still showed focal area of myocardial degeneration (Fig. 2E1). It could be noticed that the histopathological picture of the hearts from different test groups were improved without distinct variations among them.

4 Discussion

It is well documented that both cardiovascular diseases and steatohepatitis involved high oxidative stress, inflammatory condition and lipid imbalance. So, reduction of oxidative stress, inflammation and improved lipids profile could play an important role in prevention and reduction of the progression of such diseases. The nutraceuticals tested in the present study contained antioxidants, anti-inflammatory and lipid lowering bioactive constituents.

The rat model used in the present study showed elevated oxidative stress represented by the increased MDA and the reduced total antioxidant which might be a biomarker of tissue destruction and inflammation in both liver and heart. An elevated inflammatory biomarker (TNF-alpha) was also noticed in the current results. In such model significant

Fig. 1  Liver histopathology of different groups. A) Liver of control normal rat showing normal liver structure, the hepatic cords radiating from central vein separated by sinusoid (H&E X100). B-1) Liver of control DS group showing focal area of hepatic necrosis infiltrated with inflammatory cells and most of hepatocyte showing fatty degeneration (H&E X 200). B-2) Liver of control DS group demonstrated fatty degeneration, the hepatocytes taking signet ring appearance (H&E X 400). C-1) Liver of B group showed multi focal areas of fatty degeneration and focal aggregation of inflammatory cells (H&E X 100). C-2) Liver of B group showed proliferation of fibers connective tissue in portal area with inflammatory cells infiltration and fatty degeneration of hepatocytes (H&E X 200). D-1) Liver of B + T + TC group showing marked improvement of histopathological picture (H&E X 200). D-2) Liver of B + T + TC group showing marked improvement of histopathological picture (H&E X 400). E-1) Liver of fish oil group showing mild vacuolar degenerative changes while most of cells are intact (H&E X 100). E-2) Liver of fish oil group showing marked improvement in the histopathological changes (H&E X 400). F-1) Liver of F + T + TC group showing marked improvement in histopathological picture (H&E X 100). F-2) Liver of F + T + TC group showing marked improvement in histopathological picture (H&E X 400). F-3) Liver of F + T + TC group showing focal area of vacuolar degeneration (H&E X 200). B: Borage oil; F: Fish oil; T: Turmeric; TC: Alpha-tocopherol, DS: Dyslipidemic and steatohepatitis.
dyslipidemia along with elevated T.Ch/HDL-Ch emphasized the induction of cardiovascular disease according to Yang et al.\(^{34}\). Also high hepatic fat with increased inflammatory biomarker confirm the induction of steatohepatitis. The biochemical changes in such model were supported by histopathological changes in liver and heart. Induction of this model was implemented by feeding high fructose-high saturated fat diet supplemented by cholesterol and sodium cholate and deficient in fibers. Fructose stimulates hepatic lipid synthesis and block fat oxidation\(^{35}\) thereby increase hepatic deposition of fat. Plasma triglycerides were reported to increase due to fructose moiety of sweetened beverages\(^{36}\). High intake of fructose and saturated fat produced insulin resistance, dyslipidemia, high oxidative stress, fatty liver and inflammatory state\(^{3}\,^{37}\). The presence of cholic acid and cholesterol in the diet increased cholesterol absorption with consequent hypercholesterolemia. The changes in endothelial function via saturated fat intake, oxidative stress and inflammation promote initiation and induction of cardiovascular diseases\(^{38}\). Also absence of fibers in the diet could promote induction of dyslipidemia, cardiovascular disease and steatohepatitis.

Borage seed oil, besides containing 17-28% gamma linolenic, it contains palmitic (10-11%), stearic (3.5-4.5%), oleic (16-20%), linoleic (35-38) as reported previously\(^{14}\) which coincided with the results of the current study. In addition borage oil showed the presence of alpha-linolenic (4.559). The main bioactive fatty acids in fish oil identified from GC analysis were EPA (22.768) and DHA (13.574) which were similar in concentration to that reported previously\(^{39}\) which could be ascribed to the different origin of fish oil. Generally, all nutraceuticals used in the present study produced improvement of both biochemical and histopathological changes. Treatment by either fish or borage oil alone restored the normal levels of plasma T.Ch, LDL-Ch, T.Ch/HDL-Ch and liver fat. HDL-Ch was only normalized on treatment with fish oil. Plasma TAC was normalized on ad-

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**Fig. 2** Heart histopathology of different groups. A) Heart of control normal rat showed normal histological structure (H&E X 400). B1) Heart of control DS group demonstrated focal areas of regenerative changes with loss of cardiac myofibres striation and congestion of cardiac blood vessels (H&E X 400). B2) Heart of control DS group exhibited pericardial edema and separation of myofibers and inflammatory cells infiltration (H&E X 200). C1) Heart from B group showed focal area of myocardial edema with inflammatory cells infiltration (H&E X 200). C2) Heart from B group demonstrated improvement in histopathological changes (H&E X 200). D1) Heart from B + T + TC group showing marked improvement in the histopathological picture (H&E X 200). D2) Heart from B + T + TC group showing marked improvement in the histopathological picture (H&E X 400). D3) Heart from B + T + TC group still showed loss of striation of cardiac muscles (H&E X 400). E1) Heart from fish oil group exhibited mild myocardial degenerative changes (H&EX200). E2) Heart from fish oil group showed marked improvement of myocardial fibers (H&E X400). F1) Heart from F + T + TC group showing focal area of myocardial degenerative (mild) (H&E X 400). F2) Heart from F + T + TC group showing marked improvement in the histopathological picture (H&E X 200). B: Borage oil; F: Fish oil; T: Turmeric; TC: Alpha-tocopherol, DS: Dyslipidemic and steatohepatitis
ministration of any nutraceutical except for fish oil. MDA level matches that of normal control on treatment with the different nutraceuticals while borage oil supplemented by turmeric and tocopherol showed lower level than normal pointed to high antioxidant effect of such nutraceutical that could be ascribed not only to the antioxidant activity of T and TC but also to that of gamma-linolenic. Liver total fat was significantly reduced on treatment of the different nutraceuticals; while it was only normalized on treatment with fish oil and borage oil.

Gamma linolenic acid from borage oil is converted into the body to dihomo gamma linolenic acid, a precursor of 1-series prostaglandins and 3-series leukotriene, thereby inhibit leukotriene synthesis resulting in anti-inflammatory and antithrombotic effect. Borage oil holds great promise for modulating inflammatory disorders. So, it can prevent the incidence of cardiovascular disease. Borage oil possess anti inflammatory activity which is superior compared to gamma-linolenic itself. Borage oil reduced progression of cardiac remodeling after myocardial infarction and congestive heart failure in rats through attenuation of inflammatory infiltration and fibrosis in the myocardium as could be seen from histopathology examination of the heart and the reduction in TNF- alpha in the present study. Gamma linolenic acid afforded protection from ventricular fibrillation in aged rats.

Fish oil and borage oil were reported previously to reduce TCh, TGs and hepatic lipids in mice which agreed with the current research. Oleic acid a monounsaturated fatty acid which is present as 20.163 and 12.771% in borage and fish oil, respectively were reported to have cardioprotective effect via improving vascular endothelial function. The cardiac and hepatic ameliorating effect of fish oil seen in the present study can be justified based on its content of the anti-inflammatory ω3 fatty acids DHA and EPA via an effect on cytokines. Despite the high unsaturation of fish oil fatty acids however it showed antioxidant effect in the present study which agreed with the previous works. EPA was reported as potent antioxidant due to its distinct lipophilic and electron stabilization property. EPA also improves HDL function represented by enhancing cholesterol efflux, anti-inflammatory and antioxidant effect. Both DHA and EPA possess beneficial effect towards cardiovascular health, promoting vascular smooth muscle relaxation and vasodilatation. The low n-6: n-3 fatty acids ratio in fish oil compared to borage oil might add to the health benefit of fish oil in the present study.

Monounsaturated fatty acids (20.163% in borage oil and 22.12% in fish oil) were demonstrated to reduce hepatic deposition of fat due to their insulin sensitizing effect. Fish oil was reported to stimulate gene expression related to fatty acids beta oxidation with simultaneous reduction of those induce lipogenesis in liver and possess hepatoprotective effect. Omega3 polyunsaturated fatty acids activate the peroxisome proliferator receptor (PPAR) alpha in liver thereby stimulate fatty acid oxidation with simultaneous activation of PPAR gamma that lead to increases insulin sensitivity, reduction of hepatic lipogenesis and reduced reactive oxygen species with consequent reduction of hepatic fat and slowing down the progression to steatohepatitis. This could reflect the significant reduction of liver fat in the present work on treatment with fish oil. Increased insulin sensitivity on administration of fish oil is an important issue because insulin resistance is one of the major proposed mechanisms involved in all stages of non alcoholic fatty liver disease. Elevated cytokines, as inflammatory biomarker, have strong link to steatohepatitis, so reduction of them by omega3 fatty acids is essential in the management of such disease.

It was confirmed that alpha and gamma-linolenic acids increased fatty acid oxidation in the liver while they differ considerably in how they affect individual fatty acid oxidation enzyme. Gamma-linolenic acid enhanced the activity of carnitine palmitoyl transferase and peroxisomal beta oxidation that demonstrated reduction of body fat including that of liver due to fatty acid beta-oxidation.

Combination of antioxidants (turmeric and tocopherol) with either borage or fish oil in the present study showed marked reduction of the effect of both oils on liver fat without complete abolishing. This effect is difficult to be explained however it might be related to some sorts of interaction or an effect on biotransformation enzyme in the liver that might have an impact on the metabolism of both gamma-linolenic and omega-3 polyunsaturated fatty acids. On the other hand, curcumin was demonstrated to combat liver fatty acid oxidation; thereby might antagonize the reported effect of both fish oil and borage oil as enhancer of fatty acid beta-oxidation.

Inclusion of turmeric and vitamin E within nutraceuticals did not add any beneficial effect concerning biochemical parameters except in few cases like the significant increase in TAC in case of fish oil containing turmeric and vitamin E compared to fish oil as well as the significant reduction in MDA and more improvement in liver histopathology on administration of borage oil combined with turmeric and vitamin E compared to borage oil. The elevation of TAC and reduction of MDA are expected due to the antioxidant activity of both vitamin E and turmeric. Vitamin E was reported to stabilize mitochondrial function and protect the liver against oxidative damage with consequent anti-inflammatory activity thereby could prevent progression of fatty liver to steatohepatitis.

The health benefit of turmeric is certainly attributed to curcuminoids contents especially curcumin determined in the present study. Curcumin is metabolized in the body to tetrahydrocurcumin that possess antioxidant property and improved cardiac hypertrophy in rats. Curcumin was re-
ported to alter serum peroxidation as could be seen in the present study. Also turmeric was demonstrated to contain bioactive essential oil and polyphenol of reported remedial anti-inflammatory effect. Vascular endothelial function which is a potential risk of cardiovascular disease was improved by curcumin supplementation via anti-inflammatory and antioxidant mechanism. Curcumin improved dyslipidemia and liver dysfunction in high fat high sucrose fed rats. Curcumin has the ability to alleviate myocardial injury with inhibition of pro-inflammatory cytokine in rats with coronary artery ligated and in isolated heart which was likely demonstrated in the present study. Modulation of post-translational modifications (like protein acetylation) is considered as one of the most recent strategy to combat cardiovascular disease; curcumin and DHA are emerging agents that possess this activity mediated through modulation of sirtuin. This is because protein hyperacetylation is associated with different cardiovascular diseases.

The non significant change in body weight gain among the different experimental groups with simultaneous reduction in total food intake in rats fed on DS (control and test groups) compared to NC fed on balanced diet could be explained on the basis of the high Caloric contents of DS compared to balanced diet due to high fat percentage. The increase in liver/body weight % in DS compared to NC might be attributed to the increased hepatic fat deposition while the reduction in liver/body weight % on administration of different nutraceuticals is related to reduction of liver fat.

5 Conclusion
Borage and fish oil possess equal protective effect towards cardiovascular and fatty liver diseases except for liver histopathological changes which was more efficiently improved by fish oil and the superiority in improving TAC in case of borage oil treatment.

Inclusion of turmeric and vitamin E within nutraceuticals did not add any beneficial effect concerning biochemical parameters except in few cases like the significant increase in TAC in case of fish oil containing turmeric and vitamin E compared to fish oil. Another exception is the significant reduction in MDA and more improvement in liver histopathology on administration of borage oil combined with turmeric and vitamin E compared to borage oil.

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Conflict of interest statement
The authors declare that they have no conflict of interest.

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