The fish oil rich in Omega-3 fatty acids, the Nigella sativa oil rich in thymoquinone and the Sea Buckthorn fruit are known for their antioxidant, anti-inflammatory and anti-obesity properties. Due to these properties the study aimed to make an association between each remedy and its corresponding target tissue where the beneficial effects of the supplement is maxim. Thirty female adult Wistar rats were kept on high/caloric high fat diet (30%) and divided into five groups according to the above-mentioned supplements administered concomitantly with the diet for six weeks, as follows: Cod oil fish (EPA and DHA 0.1 g/kg), Nigella Sativa oil (0.1 g/kg), and Sea Buckthorn fruit 1 g/kg. Histopathological exam of their brain, heart, and kidney was performed on their tissues and correlated with the usual plasma biological parameters and erythrocyte antioxidant enzymes. Regarding their metabolic effects, fish oil had the strongest lipid-lowering effect, and at the same time increased the HDL cholesterol significantly. The Sea buckthorn fruit corrected the high blood glucose levels, increased the erythrocyte SOD activity, while the Nigella Sativa oil increased the erythrocyte GPx activity. In conclusion, by comparing the three supplements - Nigella sativa oil, fish oil and Sea Buckthorn fruit - the best neuroprotective effect was observed in the fish oil, the best renal protector was the Sea buckthorn fruit and a relative cardio protective effect was provided by Nigella sativa and Sea buckthorn supplements.

Keywords: high fat diet, antioxidants, inflammation, Fish oil, Nigella sativa, Sea buckthorn
with standard chow (STD group), the group who continued with high fat diet without any supplement (FAT group), the group on high fat diet associated with fish oil rich in Omega-3 fatty acids - O group (0.1ml/kg/day), the group on high fat diet associated with Nigella Sativa oil - N group (0.1ml/kg/day) and the group on high fat diet associated with Sea Buckthorn fruit 1g/kg/day the F group. One group, the control group was raised during all the experiment on standard chow (CON group, n=6).

The rats were sacrificed by cervical dislocation and the tissues: brain, kidney and heart were harvested for histopathological assessment. Blood samples from carotid arteries were obtained for the following parameters: triglycerides, total cholesterol, HDL-c, uric acid, albumin, HDL-c, glucose, urea and total proteins. These measurements were done by using Hospitex Diagnostics kits, Romania). SOD and GPx were measured by Elisa methods, by using Immunodiagnostics kits, Ref K7120 catalog and Ref 30-7031 catalog, respectively. The experimental procedures were carried out under Convention 86/609/E.C.C. from November 24, 1986, for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes.

Fresh samples of tissues were fixed in 10% formalin for at least 24 h. After paraffin-embedding, the samples were cut into 5µm-thick sections using a rotary microtome and stained with Hematoxylin–Eosin. The histopathological slides were examined and images were acquired using a Carl Zeiss Jena photomicroscope.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>O</th>
<th>N</th>
<th>F</th>
<th>FAT</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein [g/dL]</strong></td>
<td>7.3±0.89</td>
<td>7.28±0.65</td>
<td>6.7±0.77</td>
<td>6.91±0.86</td>
<td>7.27±0.91</td>
</tr>
<tr>
<td><strong>Albumin [g/dL]</strong></td>
<td>3.55±0.45</td>
<td>3.58±0.48</td>
<td>3.3±0.45</td>
<td>3.65±0.58</td>
<td>3.55±0.38</td>
</tr>
<tr>
<td><strong>ALT [U/L]</strong></td>
<td>48.3±0.76</td>
<td>55.26±0.59</td>
<td>64.5±0.78</td>
<td>97.82±11.04</td>
<td>63.48±7.55</td>
</tr>
<tr>
<td><strong>AST [U/L]</strong></td>
<td>10.14±1.15</td>
<td>20.11±3.20</td>
<td>20.96±2.51</td>
<td>26.19±3.08</td>
<td>10.38±12.65</td>
</tr>
<tr>
<td><strong>Uric acid [mg/dL]</strong></td>
<td>1.38±0.14</td>
<td>1.72±0.11</td>
<td>1.65±0.14</td>
<td>2.22±0.31</td>
<td>1.89±0.17</td>
</tr>
<tr>
<td><strong>Glucemia [mg/dL]</strong></td>
<td>135.67±16.81</td>
<td>121.01±14.71</td>
<td>113.2±12.97</td>
<td>155.78±14.89</td>
<td>104.96±11.96</td>
</tr>
<tr>
<td><strong>Cholesterol [mg/dL]</strong></td>
<td>40.15±6.34</td>
<td>43.21±4.77</td>
<td>51.22±5.35</td>
<td>71.5±10.38</td>
<td>40.12±6.24</td>
</tr>
<tr>
<td><strong>Triglycerides [mg/dL]</strong></td>
<td>57.89±17.22</td>
<td>125.13±18.98</td>
<td>87.25±19.98</td>
<td>150.93±17.66</td>
<td>78.4±8.98</td>
</tr>
<tr>
<td><strong>Urea [mg/dL]</strong></td>
<td>45.4±7.36</td>
<td>32.36±3.87</td>
<td>50.65±6.12</td>
<td>50.5±7.07</td>
<td>34.57±5.12</td>
</tr>
<tr>
<td><strong>HDL-c [mg/dL]</strong></td>
<td>32.4±2.31</td>
<td>25.4±3.11</td>
<td>29.6±3.76</td>
<td>15.9±1.96</td>
<td>28.4±3.41</td>
</tr>
<tr>
<td><strong>SOD [U/g Hb]</strong></td>
<td>110.81±11.21</td>
<td>116.52±14.39</td>
<td>131.66±14.32</td>
<td>94.72±11.75</td>
<td>105.6±11.45</td>
</tr>
<tr>
<td><strong>GPx [U/g Hb]</strong></td>
<td>8.27±0.94</td>
<td>9.45±1.87</td>
<td>8.4±0.96</td>
<td>7.8±0.96</td>
<td>7.5±0.96</td>
</tr>
</tbody>
</table>

T-test, p values were obtained by the comparison of the FAT group with the other groups, p<0.05 for ALT between FAT and O group; p<0.05 for glucemia between FAT and F; p<0.05 for triglycerides between FAT and O; p<0.05 for HDL between FAT and O; p<0.05 for Cholesterol between FAT and N; p<0.05 for uric acid between FAT and O; p<0.05 for SOD between FAT and F; p<0.05 for GPx between FAT and N.

### Histopathological exam

**O Group**

On the cerebellum level the main observation consists of nonspecific vacuolation in the molecular layer, Purkinje neurons with focal moderate basophil cytoplasm and condensations of nuclear chromatin. N group: normal appearing cortex, the cerebellar molecular layer shows the limited areas where vacuolisation is nonspecific. F group: Focal necrosis inside Purkinje neuron areas. In the cortex, microscopic lesions have not been identified. FAT group: cerebellar Purkinje neurons in the intermediate layer show intensely basophilic cytoplasm (necrosis) accompanied by perineural edema, and nonspecific vacuolisation on the molecular layer. The white substance of the cerebellum shows hemorrhage with limited stretch. In cortex - focal ectasia on blood vessels with moderate perivascular edema, and neurons with intensely basophilic cytoplasm. The cerebellum molecular layer shows nonspecific vacuolisation of the nerve substance. STD group: cerebellum molecular layer shows nonspecific vacuolisation of the nerve substance.

**N Group**

**F Group**

**FAT Group**

**STD Group**

**CON Group**

---

Fig 1. The histopathological aspect of brain Hematoxylin-Eosin dye, ob 40 x for: O group (with Omega 3 fatty acid oil as supplement), N group (with Nigella sativa oil supplement), F group (with Sea buckthorn fruit), FAT group (fat diet without Supplement), STD group (standard diet), CON group (raised only on standard diet).
Results and discussions

Although most studies regarding diet recommend fish as a source of Omega-3 polyunsaturated fatty acids for a healthy lifestyle, Omega-3 acids supplements in capsule represents an alternative, having the effect of preventing cardiovascular complications, by obvious lipid-lowering effects. In the literature there are many studies on the metabolic effects of Omega-3 fatty acids, but although their beneficial effect is unambiguous on lowering the triglycerides, their effect on HDL-c is controversial [15].

In our study, the females on high fat diet, without remedy, had the highest values for blood glucose, triglycerides, uric acid and transaminases (AST, ALT) activity. Among the remedies used, fish oil had the strongest lipid-lowering effect, decreasing the triglycerides significantly (p<0.03). Furthermore, the polyunsaturated fatty acids in fish oil increased the HDL cholesterol significantly (p<0.05). Sea buckthorn fruit corrected the high blood glucose levels, increased the erythrocyte SOD activity (p<0.05), while the Nigella sativa oil increased the erythrocyte GPx (p<0.05) activity. The fish oil had a broad effect on the metabolism, lowering also uric acid and ALT activity (p<0.05).

In an experimental study, the rats who were kept on a high caloric/high fat diet for 8 weeks (1% cholesterol) became dyslipidemic, with high levels of total LDL-c and decreased total plasma antioxidant activity. The treatment with thimoquinone (20 and 100 mg/kg) induced hepatic gene expression of antioxidant enzymes (SOD, GPx, catalase) improving the antioxidant defence both in the liver and in the plasma. Also, the lipid profile was improved [16].

In our study, the myocardium shows intense oxifile myocardocytes, separated by edema, fragmented, accompanied by an inflammatory infiltrate, hyperemia. N group: Intense oxifile myocardocytes separated by discreet edema, rarely fragmented, accompanied by a discreet inflammatory infiltrate, hyperemia. F group: Rare oxifile myocardocytes, accompanied by sarcoplasmic homogenization, hyperemia and interstitial inflammatory mononuclear cell infiltrate. FAT group: Cardiomyocytes separated by edema, some show homogeneous sarcoplasm, oxifila, rarely fragmented cardiomyocytes. Perivascular and interstitial mononuclear inflammatory cell infiltration (with lymphocytes, macrophages and mast cells), hyperemia. Subepicardial adipose tissue is well represented. STD group: isolated areas where cardiomyocytes are separated by edema, some areas show homogeneous sarcoplasm with oxifila. Discreet interstitial mononuclear inflammatory cell infiltrate.
oil associated to the high fat diet reduced the increased levels of cholesterol as it shown in the N group versus the FAT group. The cholesterol-lowering effect of Nigella sativa oil is explained by the fact that this oil is rich in phytosterols, effective in inhibiting the absorption of dietary cholesterol [18].

In a clinical study done in 62 patients with metabolic syndrome, who received 3 g of Omega-3 fatty acids per day, for one year, it was observed a decrease in triglycerides, without significant change in HDL-cholesterol. In another study, involving nearly 2100 patients with triglycerides between 200 and 500 mg/dL, the decreasing effect on triglyceride level was comparable between the groups treated with Omega-3 as monotherapy and those treated with Omega-3 and statins [19]. In our study, the only supplement which could improve significantly the HDL level, was the cod fish oil.

MARINE and ANCHOR clinical trials [20] have shown that EPA at a dose of 4 g/day decreased triglycerides and total cholesterol, without changing the value of LDL-c. In vitro comparative studies on the effects of fatty acids EPA and DHA it was demonstrated that EPA is the most potent inhibitor of LDL oxidation, and has direct anti-oxidant action [21]. In our study, in group O versus FAT group, the intake of Cod fish oil (0.1mg/kg rat) of both EPA and DHA (147.2 mg EPA/ml fish oil and 92 mg/ml fish oil) for 6 weeks decreased the increased level of triglycerides but did not influence significantly the erythrocyte antioxidant enzymes, SOD and GPx.

Quercetin, a flavonoid present in Sea buckthorn berry, in addition to its antioxidant effects, has lipid-lowering effect. Fibers and polyphenols in Sea buckthorn extract residue improve the post-prandial lipemia [22]. It is known that the Sea buckthorn berry is rich in flavonoids and vitamin C which have anti-inflammatory effects and act through a synergic mechanism [23].

In rats treated with diet rich in sugar, Sea buckthorn seed flavonoids improved dyslipidemia and hyperinsulinemia [24]. In streptozotocin-induced diabetic rats, the aqueous extract of Sea buckthorn seeds, rich in flavonoids, reduced blood glucose, triglycerides, and increased the activity of serum SOD and blood glutathione [25].

Clinical studies have shown that Sea buckthorn fruit and alcoholic extract from the fruit of Sea buckthorn reduced post-prandial blood glycaemia and improved insulinemia values. [26]. In our study, we gave Sea buckthorn berry for six weeks, 1g/kg rat/day and we observed that in the F group versus the FAT group, the glycaemia was normalised and the erythrocyte SOD activity increased significantly.

In the histopathological images, we analysed the appearance of the cells, degenerative changes and other lesions and also the inflammatory infiltrations in order to evaluate the potential of the three remedies to counter the damage of a high fat diet.

In our study, the rats from the FAT group had granular tubulonephrosis, associated with inflammation. All three remedies improved renal histopathologic appearance, protecting renal cells, reducing inflammation and preventing the occurrence of vacuolation. Of the three remedies, the highest anti-inflammatory effect was observed in the Sea buckthorn group. In the standard group (without any remedy), the inflammatory infiltration persisted, along with granular dystrophy.

The females from the FAT group had severe brain changes as necrosis, haemorrhage and edema. From the all remedies, the fish and Nigella sativa oils protected the cortex and the Sea buckthorn fruit had lower cerebral protective effects. Our results are in accordance with other studies. It was demonstrated that neuronal apoptosis and neurodegenerescence lesions is usually associated with inflammation and with a low local antioxidant defence. [27, 28]. Polyunsaturated fatty acids act as anti-oxidants, protecting neuronal cell membranes from oxidative damage and as anti-inflammatory mediators in the brain [29].

In our study, the fatty diet caused dilacerations in myocardial cells, swelling, and inflammation as is shown in the FAT group. The treatment with Nigella sativa oil and Sea buckthorn fruit gave the best results in protecting the myocardium, even if it couldn’t reverse the injuries.

Obesity is associated with: dyslipidemia, oxidative stress, a moderate inflammatory status and high values of uric acid. The determination of inflammatory markers and oxidative stress parameters in obesity should help the physicians to get a panoramic view of the metabolic profile and apply a personalized management. As examples, there were studies which demonstrated that obese older women with metabolic syndrome are more prone to have a severe inflammatory status [30]. Furthermore, another study showed that subjects with metabolic syndrome and high levels of uric acid have increased concentration of inflammatory parameters such as C-reactive protein. [31]

We aimed to find the ideal supplement which corrects at once the cluster pathogenic modifications.

Conclusions

By comparing the anti-inflammatory, antioxidant and metabolic effects of the three remedies on kidney, brain and heart, analysing histopathologic aspects of these tissues and observing the biological parameters level, we can conclude that each supplement has a target tissue. This opens new possibilities for physicians to apply a personalised management in obesity.

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