Tissue Protective Effects of Three Supplements: Nigella Sativa Oil, Fish Oil and Sea Buckthorn Fruit, in High Caloric/High Fat Diet

A comparative study

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The fish oil rich in Omega-3 fatty acids, the Nigella sativa oil rich in thymoquinone and the Sea Buckthorn fruit rich in flavonoids, especially quercetin, are known for their antioxidant, anti-inflammatory and antiobesity 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 1g/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

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 and anti-inflammatory effects.

Omega-3 fatty acids activate the transcription factors peroxisome proliferator activated receptors-α (PPARα) and -γ (PPARγ). PPARα increases fatty acid oxidation and down-regulates proinflammatory genes [1] and PPAR-γ is involved in insulin sensitivity by increasing the expression of adiponectin gene [2]. Lipogenesis is reduced under the treatment with Omega-3 by inhibiting the expression of the transcription factor, sterol response element binding protein-1c, SREBP-1c [2]. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) exerts strong anti-inflammatory effects by inhibiting NFκB activity and by serving as precursors for potent anti-inflammatory lipids called resolvins and protectins [3].

The anti-inflammatory effect on Nigella sativa is due to thymoquinone that has many effects: it inhibits the enzymes cyclooxygenase and lipoxygenase, it decreases lipid peroxidation [4] and it inhibits NF-êB activity [5]. Furthermore, it also has the effect of stimulating macrophage activity and decreasing macrophage infiltration [6].

Nigella sativa antioxidant capacity is demonstrated by up-regulation the antioxidant enzymes: superoxid-dismutase (SOD), catalase and glutathione peroxidase (GPx) and by lowering the levels of nitric oxide, malondialdehyde and protein carbonyls [7]. Nigella sativa inhibits also serine-threonine kinase phosphorylation, improving insulin sensitivity [8].

Sea buckthorn decreases lymphocyte proliferation and inhibits T cell activation [9], reduces C-reactive protein and reduces the risk of cardiovascular disease [10]. Phenolic compounds and flavonoids from Sea Buckthorn fruit ameliorated bodyweight, blood glucose, and serum lipid profiles. The fibres and polyphenol from the berry delay the postprandial lipemia [11].

Organs involved in flavonol metabolism and excretion, including kidneys and liver, contain significantly higher flavonol concentrations than plasma. Thus, these organs should be considered as primary targets of potential beneficial effects [10].

This study aimed to make an association between each remedy and its corresponding target tissue where the beneficial effects of the supplement are maxim. Below we mention general bioavailability information about the study supplements.

It is well known that a diet higher in fat increases Omega-3 bioavailability and the absorption efficiencies for EPA in the triglyceride is 90% [12]. Thymoquinone has a bioavailability lower than 60%, a very low absorption but rapid elimination following oral delivery [13]. Studies concerning the delivery of the lipid compounds to the brain must take into consideration the numerous protective natural barriers surrounding the central nervous system (CNS) such as the blood–brain barrier (BBB). [14]

Experimental part

Materials and methods

Thirty female Wistar rats (age 3 weeks, 50-60 g) from Animal Facility of Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, were raised on high caloric/high fat diet (30%) until maturity (10 weeks age , 250-280g) and then for the next 6 weeks they were divided in five groups (n=6 rats/group): the group who continued

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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 Immundiagnostik kits, Ref K7120 catalog and Ref 30-7031 catalog, respectively.

The experimental procedures were carried out under Convention 86/609/E.E.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.

Parameter	Group				
	O	N	F	FAT	STD
Total proteins [g/dL]	7.31±0.89	7.28±0.65	6.78±0.77	6.81±0.86	7.27±0.91
Albumin [g/dL]	3.55±0.45	3.58±0.48	3.33±0.45	3.63±0.58	3.55±0.38
ALT [UI/L]	48.38±0.76[a]	55.26±0.59	64.50±0.78	97.82±11.04[a]	63.48±7.55
AST [UI/L]	10.14±1.15	20.11±3.20	20.96±2.51	26.19±3.08	10.38±12.65
Uric acid [mg/dL]	1.38±0.14[b]	1.72±0.11	1.65±0.14	2.21±0.31[b]	1.89±0.17
Glycaemia [mg/dL]	135.67±16.81	121.01±14.71	113.21±12.97[c]	155.28±14.80[c]	104.96±11.86
Cholesterol [mg/dL]	49.18±6.34	43.21±4.77[d]	51.22±5.35	71.33±10.28[d]	40.12±6.24
Triglycerides [mg/dL]	57.89±17.22[e]	125.13±18.98	87.25±19.98	150.93±17.66[e]	78.40±18.98
Urea [mg/dL]	45.47±5.36	32.36±3.87	50.65±6.12	50.51±7.07	34.57±5.12
HDL-c [mg/dL]	32.4±2.31[f]	25.4±3.11	29.0±3.76	15.9±1.96[f]	28.8±3.41
SOD [U/g Hb]	110.81±11.21	116.5±14.19	131.66±14.32[g]	94.72±11.75[g]	105.6±11.45
GPx [U/g Hb]	8.27±0.94	9.45±1.87[h]	8.4±0.96	7±0.89[h]	7.5±0.96

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 glycaemia between FAT and F; $p<0.03$ 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.

Table 1
SERUM BIOLOGICAL PARAMETERS OF RATS

Histopathological exam

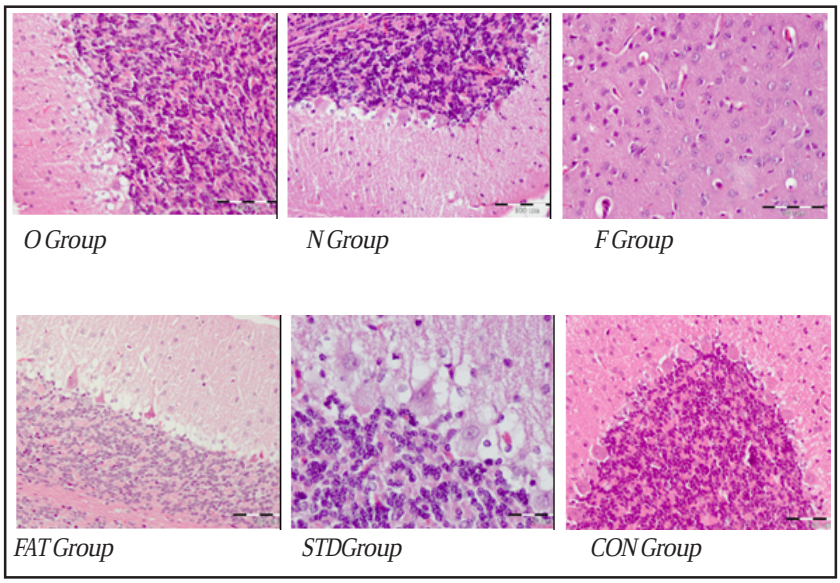
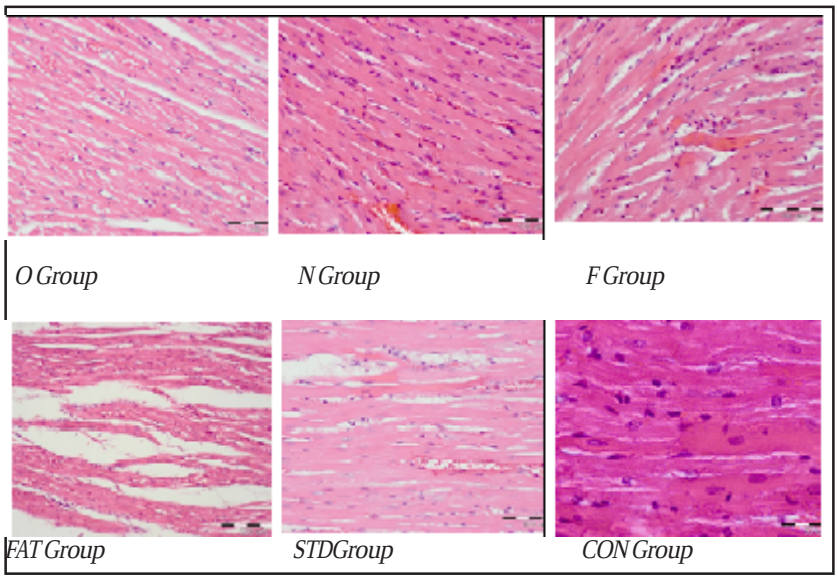


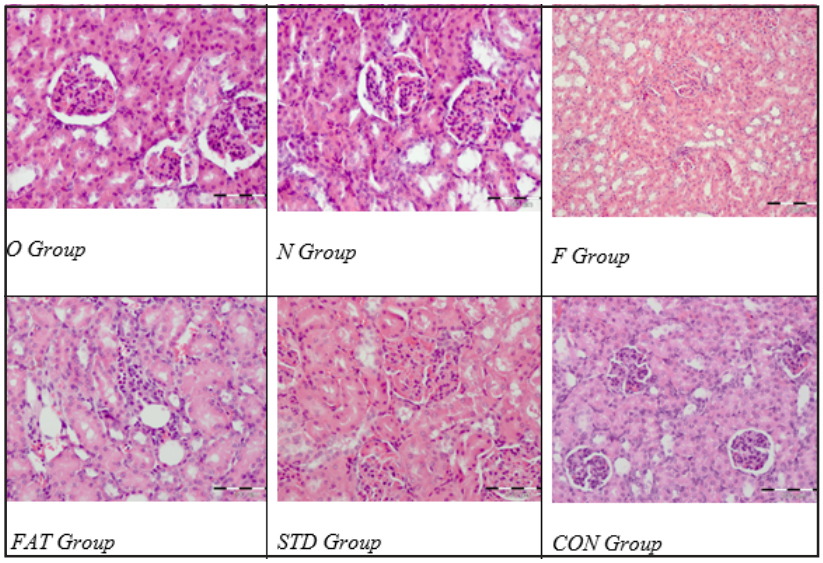
Fig 1.The histopathological aspect of brain Hematoxilin-Eosin dye, ob 40 x for:O group (with Omega 3 fatty acid oil as supplement), N group (with Nigella sativa oil supplement), F group (with Sea buckthorn fruit), FAT group (fat diet without Suplemment), STD group (standard diet), CON group (raised only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

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.



O group: myocardium shows intense oxifile myocardiocytes, separated by edema, fragmented, accompanied by a inflammatory infiltrate, hyperemia. N group: Intense oxifile myocardiocytes separated by discreet edema, rarely fragmented, accompanied by a discreet inflammatory infiltrate, hyperemia. F group: Rare oxifile myocardiocytes, 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.

Fig 2.The histopathological aspect of heart Hematoxilin-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 Suplemment), STD group (standard diet), CON group (raised only on standard diet)



O group: focal in the cortical collector tubules there are degenerative changes (granular tubulonephrosis). Hyperemia at both mesangial and interstitial level. N group: passive focal hyperemia observed in the glomeruli, accompanied by increased filtering space. Interstitial hyperemia and discrete perivascular edema, focal granular tubulonephrosis. Moderate interstitial inflammatory cell infiltration, consisting of lymphocytes, macrophages, neutrophils. F group: focal degenerative changes in the cortical collector tubules (granular tubulonephrosis). Hyperemia at both mesangial and interstitial level. FAT group: numerous degenerative changes in the cortical collector tubules, vacuolar/granular tubulonephrosis, often accompanied by tubulointerstitial necrosis. Hyperemia at both mesangial and interstitial level. Increased glomerular filtration space. Interstitial multifocal inflammatory infiltrate composed of lymphocytes, macrophages, neutrophils and rare eosinophils. STD group: epithelial cells of the tubules often have degenerative lesions (granular dystrophy). Glomerular vascular ectasia. Discreet stromal mononuclear inflammatory infiltrate.

Fig 3.The histopathological aspect of kydney Hematoxilin-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 Suplemment), 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 with 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]. A clinical study done in high blood pressure patients versus control, showed that the administration of Nigella sativa oil for eight weeks decreased the amount of LDL cholesterol and improved the blood pressure levels, both systolic and diastolic [17]. In our study, Nigella sativa

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 trough a synergic mechanism [23].

In rats treated with diet rich in sugar, Sea buckthorn seed flavonoids improved dyslipidaemia 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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