https://doi.org/10.31925/farmacia.2024.3.12 **ORIGINAL ARTICLE**

OUTCOME OF CO-ADMINISTRATION OF SEA BUCKTHORN FRUIT IN HIGH FAT DIET WISTAR RATS

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Manuscript received: January 2024

Abstract

Obesity is a risk factor for diabetes, cardiovascular diseases, cognitive impairment and sarcopenia. Sea Buckthorn fruit may have beneficial effects on muscle mass and memory. This study evaluated the metabolic impact of a high-fat diet on young Wistar rats and the effects of co-administering Sea Buckthorn fruit on skeletal muscle and brain tissues. Rats aged 10 weeks were divided into three groups (n = 6 per group) and for the next 8 weeks were raised on: standard diet (Control group), high-fat diet (HFD group) and high-fat diet with Sea Buckthorn fruit (0.3 g fruit/100g rat weight/day), (HFDSB group). In the HFD group, the gastrocnemius muscle showed decreased phosphorylation of Akt protein (pAkt 5.77%) and the haematoxylin-eosin staining demonstrated brain and muscle pathological changes. Serum tests indicated metabolic imbalances. The co-administration of the Buckthorn fruit to the high fat diet reduced the intervisceral fat, glycaemia ($p < 0.05$), triglyceridemia ($p < 0.05$) and resistininemia ($p < 0.05$). It also increased the insulin sensitivity (pAkt 21.2%, $p < 0.05$) and the tissue histological appearance for the brain and for the muscle was almost normal. In conclusion, Sea Buckthorn fruit mitigated skeletal muscle and brain damage and enhanced insulin sensitivity in rats on a high-fat diet.

Rezumat

Obezitatea este un factor de risc pentru diabet, boli cardiovasculare, tulburări cognitive și sarcopenie. Fructele de cătină pot avea efecte benefice asupra masei musculare și memoriei. Acest studiu a evaluat impactul metabolic al unei diete bogate în grăsimi asupra șobolanilor tineri Wistar și efectele co-administrării fructelor de cătină asupra țesuturilor musculare și cerebrale. Șobolani în vârstă de 10 săptămâni au fost împărțiți în trei grupuri (n = 6 *per* grup) și pentru 8 săptămâni au urmat diverse diete: dietă standard (grupul Control), dietă bogată în grăsimi (grupul HFD) și dietă bogată în grăsimi cu fructe de cătină, (0.3 g fruct/100g greutate șobolan/zi), (grupul HFDSB). În grupul HFD, mușchiul gastrocnemius a avut o fosforilare scăzută a proteinei Akt (pAkt 5,77%), iar țesuturile cerebrale și musculare au prezentat modificări histopatologice evidențiate la colorația hematoxilină-eozină. Testele sangvine au indicat dezechilibre metabolice. Co-administrarea fructelor de cătină la dieta grasă a redus grăsimea intervisccerală, glicemia ($p < 0,05$), trigliceridemia ($p < 0,05$) și rezistinemia ($p < 0,05$). De asemenea, a crescut sensibilitatea la insulină (pAkt 21,2%, $p < 0,05$), iar aspectele histologice cerebrale și musculare au fost aproape de normal. În concluzie, asocierea fructelor de cătină la șobolanii cu dietă bogată în grăsimi a atenuat deteriorarea mușchilor scheletici și a creierului și a îmbunătățit sensibilitatea la insulină.

Keywords: obesity, Wistar rats, Sea buckthorn, brain, insulin sensitivity

Introduction

Obesity is considered a risk factor for numerous chronic conditions such as type 2 diabetes, dyslipidaemia, cardiovascular diseases and even cognition impairment [1]. In obesity, the high reactive oxygen species are able to trigger key cellular signalling pathways, which partly explain the onset of insulin resistance and metabolic syndrome [2]. Excess feeding has been shown to increase mitochondrial reactive oxygen species production and a more oxidised state in skeletal muscle is developed [3, 4]. The high generation of the superoxide anion activates JNK protein. This protein drives insulin resistance and maintains metabolic inflammation. Activation of JNK produces serine/ threonine phosphorylation of the insulin receptor substrates which reduces phosphatidylinositide-3 kinase (PI3K) AKT signalling in response to insulin receptor activation [5]. JNK activity in macrophages promotes adipose tissue IL-6 expression, which increases adipose tissue lipolysis and circulating free-fatty acids. The high level of the long fatty acids in insulinodependent tissues is followed by increased DAG (diacylglycerol) and consecutively, insulin resistance in skeletal muscle is developed [6].

Dietary Sea buckthorn (*Hippophae rhamnoides* L.) has a positive effect on increasing muscle mass and improving muscle fibre size distribution [7]. The Sea buckthorn berries contain over 100 different phyto-chemicals such as: amino acids, vitamins, tocopherols, carotenoids, polyphenols, flavonoids, fatty acids and other active ingredients [8]. The soft part of Sea buckthorn berries is one of the richest sources of palmitoleic acid which strongly stimulates muscle insulin action [9]. Studies have shown that the effect of some proteins from the Sea buckthorn on muscles is to increase the glycogen content, increasing glycogen synthetase (GS) activity and upregulating the enzyme phosphatidylinositol 3-kinase (PI3K) [10].

Isorhamnetin, quercetin and kaempferol, the main ingredients of the flavonoid glycosides are responsible for the neurotrophic activities of this fruit, signalling *via* PI3K/Akt and ERK pathways [11, 12]. Furthermore, the flavonoids from Sea buckthorn can improve cognitive functions in high fat diet induced obese mice by hindering neuronal loss and memory impairment in behavioural tests [13]. Moreover, the same study showed that Sea buckthorn flavonoids stimulated IRS-1/Akt pathways while suppressing the NF-κB signalling and its associated inflammatory mediator expressions [13].

In a study performed on a high-fat diet treated rats it was concluded that Sea buckthorn can reduce neuro-inflammation due to its polysaccharides, by inhibiting the NF-κB pathway and also enhancing the synaptic function [14].

In clinical studies it was demonstrated that the Sea buckthorn juice improved mood and mental performance in healthy young adults, while the Sea buckthorn oil improved memory and attention in elderly patients with mild cognitive impairment [15]. In a recent experimental study it was shown that Sea buckthorn extract has antioxidant properties against immobilization stress-induced oxidative and nitrosative stress at the brain level, reversing the glutathione levels and glutathione peroxidase activities [16].

Developing novel drugs or finding supplements that ameliorate morbidities associated with obesity is an immediate priority. In our study, we aimed to demonstrate that the fresh Sea buckthorn fruit is an efficient approach for cognitive challenges associated with metabolic syndrome/obesity. This study was designed to analyse the potential beneficial role of the Sea buckthorn fruit, on two targets, skeletal muscles and brain, in high fat diet rats.

Materials and Methods

The studied groups divided by diet

Eighteen young, 10 weeks aged healthy Wistar rats were divided in three groups (n = 6 rats *per* group) according with the diet: standard chow (Control group), high fat diet without any supplement (HFD group), high fat diet associated with Sea Buckthorn fruit, 0.3 g fruit/100 g rat weight/ day (HFDSB group). There is no standard dose for Sea Buckthorn fruit as a supplement. The average weight of the rats was 350 g, so each rat received around 1.05 g of fruit/day. The high caloric, high fat diet contained 80% lipids and the thawed berry fruit was prepared each morning, for each rat as puree mixt with butter and white chocolate, so the rats ate the supplement without problems. Also, the rats had access to standard rodent chow and water *ad libitum*. They were housed in cages at 20 - 22°C temperature, humidity 50 ± 5%, 12 h cycle dark/light.

Sample collection and analysis

The rats were sacrificed by cervical dislocation and the tissues: lateral gastrocnemius muscle and brain were harvested for histopathological assessment. Blood samples from carotid arteries were obtained for the following parameters: lipid profile (triglycerides, total cholesterol, HDL-c), uric acid, albumin, glucose, urea and total proteins. Hospitex Diagnostics kits, Romania were used for the colorimetric measurements. Adiponectin and resistin were measured by Elisa methods with kits from DRG International Inc. USA, EIA 4283 kit and 4172 kit, respectively.

The EU Directive 2010/63 regarding the protection of animals was used for the experimental and other scientific purposes. The Ethics Committee of Scientific Research of "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania approved the experimental protocol, recorded as code 134/2017.

Histopathological assessment

Tissue samples of muscle and brain from all groups were fixed in 10% neutral buffered formalin (pH = 7.0) for 24 hours, followed by dehydration in: (1) 70% ethylic alcohol for 60 min, (2) 96% ethylic alcohol for 45 min and 3) absolute ethylic alcohol for 2 h. The clearing phase of the samples was made by repeated xylene immersions, embedded after in paraffin wax. The samples were automatically processed with tissue processor STP 120-3 (Thermo Fischer Sci., USA) and paraffin embedding was done with modular tissue embedding center Microm EC 350-1 (Thermo Fischer Scientific, USA). The resulting blocks were cut at 2 - 3 μm using the RM 125RTS (Leica, Germany) microtome. The sections were stained with haematoxylin and eosin (HE) by using the Microm HMS 70 (Thermo Fischer Scientific, USA) slide stainer.

The examination was performed with an Olympus BX43 microscope (Olympus, Japan) coupled to an Olympus DP73 video camera and using the Olympus Cell B analysis system for microscopic evaluation of the samples. For muscle tissue samples, longitudinal axis sections and, also, a cross sections were analysed, while for brain tissue samples, sagittal sections of right-brain hemisphere were analysed.

Immunohistochemical analysis

For immunohistochemical chromogenic evaluation, the P-Akt (Thr 308)-R antibody was obtained from rabbits in response to an amino acid sequence containing human-origin Akt protein Thr 308. The antibody is used to detect the three isoforms of pAkt (phosphorylated protein) in various species (mouse, rat, human, horse and birds). The antibody was applied to sections obtained from paraffin blocks of the studied tissue. Immunohistochemistry for the detection of Akt was performed using antibodies at a 1 in 50 dilution in commercial antibody diluent. Sections were incubated with antibody overnight, using Dako Autostainer. Control slides were incubated without primary antibody and processed similarly to the other samples. The evaluation of the IHC was performed subjectively. On multiple sections obtained from different areas of the same tissue, nuclei stained in violet (corresponding to non-phosphorylated Akt protein) were counted and compared to those stained in brown (corresponding to phosphorylated Akt protein, pAkt) under the microscopic fields. The evaluation was performed independently by two observers. In cases where nuclei showed a combination of both colours, the predominant colour intensity was considered for counting. The mean values for each of the two variants (violet nuclei and brown nuclei) were calculated.

Statistical analysis

For the Statistical analysis, GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, was used to perform the t-test. Statistical significance was set at a p-value of < 0.05.

Results and Discussion

Nowadays, there is a great interest in using medicinal plants and their bioactive compounds as supplements for obesity treatment. Sea Buckthorn fruit has hypo-glycaemic, anti-hypertensive and anti-inflammatory effects [17]. The Sea buckthorn berries were used by ancient Greeks to feed racehorses to increase their muscle mass [9]. Recently, it was demonstrated that muscle mass is associated with insulin sensitivity independent of detrimental adipose depots in young men [18]. Moreover, it was proposed that the communication between the skeletal muscle and the immune system seems to be very intense. The tight balance among them provides a proper environment not only for the skeletal muscle repair but also to improve immune system function [19].

Jana *et al*. described the molecular cascades linking cytosolic lipid excess and mitochondrial dysfunction in the pathogenesis of high fat diet-induced insulin resistance in skeletal muscle. They consider that the sequential processes following the excess intake of high fat diet in skeletal muscle includes: accumulation of cytosolic fatty acids, increased production of reactive oxygen species, mitochondrial dysfunction which leads to decreased β-oxidation and increased gluco-lipotoxicity [20].

Tardif *et al.* also emphasize that muscle mitochondrial dysfunction reduces the ability of the cell to oxidize fatty acids entering the muscle cell. This is followed by ectopic muscle lipid accumulation. This metabolic change is a potential therapeutic target to counter sarcopenic obesity [21].

In our study, the average value of the intervisceral fat in the HFD group was significantly increased. In the HFDSB group, the value of the intervisceral fat approached the weight of the chow-fed rats. We calculated also the average value of the ratio between the visceral fat/rat weight (mg/g) in the studied groups and the differences were significant (Table I).

Table I

Abdominal fat-quantitative assessment

Parameter	Control group	HFD group	HFDSB group
Visceral fat mass (g)	6.501 ± 1.4 g[1]	11.329 ± 3.7 g	6.095 ± 1.3 g[2]
Ratio between the visceral fat/rat weight (mg/g)	21.5 ± 3 g[1]	30.69 ± 4.5 g	20.8 ± 1.9 g[2]

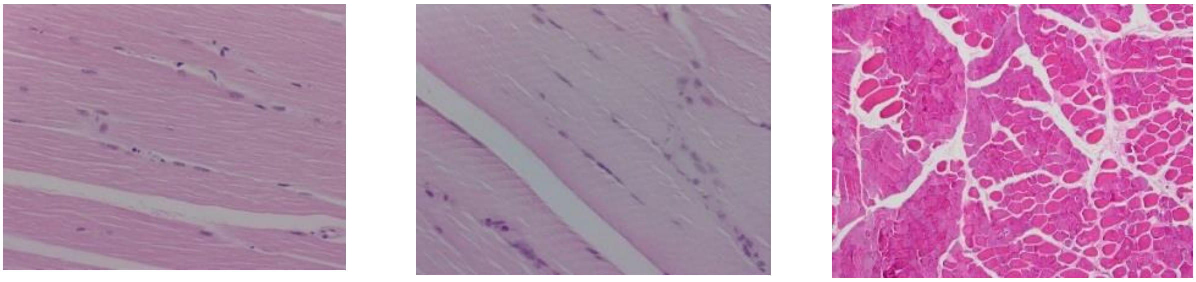
t-test, p values were obtained by the comparison of the HFD group with each other two groups; [1] $p < 0.03$; [2] $p < 0.01$

In line with our results, Sea buckthorn powder consumption in high fat diet recovered the increased body weight gain. The vicious cycle between the loss of muscle and the accumulation of ectopic fat might characterize sarcopenic obesity [22].

In our study, the haematoxylin-eosin staining of the lateral gastrocnemius muscle showed severe damage in the HFD group as the disappearance of striations and the marked oedema between the torn muscle fibres. Also, hyalinized fibres and numerous hyperchromic nuclei could be observed. In the HFDSB group, the histological appearance of the muscle is close to normal, with visible striations and isolated hyperaemia (Figure 1).

An intricate interplay of factors including: pro-inflammatory cytokines, oxidative stress, mitochondrial dysfunction, insulin resistance, dietary energy, physical activity is responsible for sarcopenic obesity [23]. Adiponectin is an adipokine with anti-diabetic, anti-atherosclerotic, anti-inflammatory effects. Adiponectin mediates these beneficial effects via direct signalling effects or *via* crosstalk with insulin signalling pathways. Obesity may induce a malfunction on adiponectin signalling [24]. The reduced circulating adiponectin in obesity is also detrimental [25].

Adiponectin, *via* the transcription factor PPARγ (peroxisome proliferator-activated receptor) signalling pathway is important in maintaining cognitive functions in chronic hypoperfusion-induced dementia [26]. There is a strong interplay between adiponectin, gut microbiota and brain-gut axis. For these reasons, researchers consider that a healthy diet rich in probiotics, prebiotics, polyphenols, can prevent many metabolic and inflammatory diseases such as neuro-degenerative diseases and obesity. Adiponectin role should be taken into account and used as a therapeutic molecule [27].



(a) Control group (b) HFD (c) HFDSB

Figure 1.

The histopathological aspect of gastrocnemius muscle haematoxylin-eosin dye, 40x

In our study, the lowest value for serum adiponectin and the highest level for resistin were measured in the HFD group. The fresh Sea buckthorn fruit reversed the low value for adiponectin and the high level for resistin to the values close to the control ones (Table II). Leptin and resistin are adipokines associated with inflammation, cardiovascular disease, insulin resistance and with food intake control. Rats fed a high fat diet exhibited significantly increased leptin and resistin levels. Researchers demonstrated that these two pro-inflammatory adipokines decreased in the groups that were provided Zeaxanthine and /or physical exercise associated to the high fat diet [28].

Table II

Serum biological parameters of rats

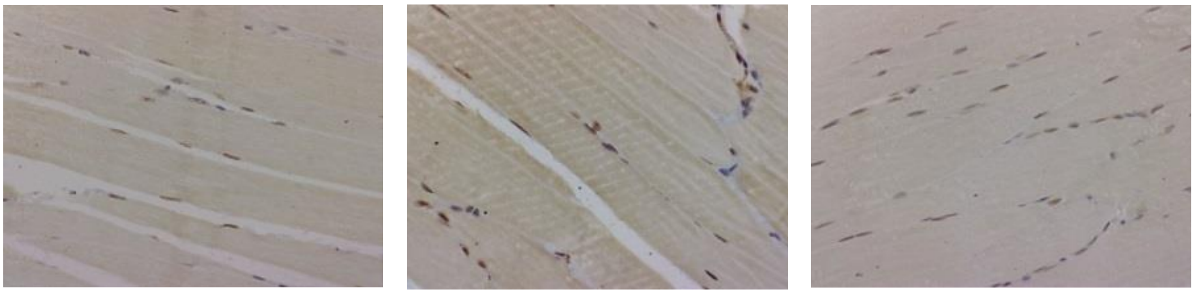
Parameter	Control group	HFD group	HFDSB group
Total proteins [g/dL]	7.3 ± 1.1	6.9 ± 0.96	6.9 ± 0.8
Albumin [g/dL]	3.6 ± 0.4	3.7 ± 0.6	3.4 ± 0.8
ALT [UI/L]	65.5 ± 8.3	98.6 ± 10.04	50.9 ± 0.9[1]
AST [UI/L]	18.8 ± 11.7	29.3 ± 4.08	19.9 ± 3.7
Uric acid [mg/dL]	1.9 ± 0.2	2.20 ± 0.51	1.51 ± 0.17
Glycaemia [mg/dL]	105 ± 12.86[2]	160 ± 15	113 ± 13[1]
Cholesterol [mg/dL]	50.2 ± 7.4	73 ± 11.2	52 ± 6.2
Triglycerides [mg/dL]	77.5 ± 20[1]	152 ± 18	62 ± 14[2]
Urea [mg/dL]	35.5 ± 5.5	51.7 ± 7.3	51.8 ± 7.2
HDLc [mg/dL]	30 ± 4.5[2]	16 ± 2.9	30 ± 3.76[2]
Adiponectin [ng/mL]	1.45 ± 0,3	1.3 ± 0.4	1.60 ± 0.3
Resistin [ng/mL]	3.3 ± 0.4[1]	4.9 ± 0.6	3.7 ± 0.5[1]

t-test, p values were obtained by the comparison of the HFD group with each other two groups; [1] $p < 0.05$; [2] $p < 0.01$

Leptin and resistin are adipokines associated with inflammation, cardiovascular disease, insulin resistance and with food intake control. Rats fed a high fat diet exhibited significantly increased leptin and resistin levels. Researchers demonstrated that these two pro-inflammatory adipokines decreased in the groups that were provided Zeaxanthine and /or physical exercise associated to the high fat diet [28].

The serine/threonine protein kinase B (PKB or pAkt) is involved in insulin-stimulated translocation of glucose transporter 4 (GLUT4), thereby facilitating intracellular glucose uptake in peripheral tissues [11]. In the present study, the immunohistochemical expression of the phosphorylated Akt protein, pAkt, in the skeletal muscle tissue was assesed. Observing the results in the Figure 2, the lowest percentage of pAkt protein (5.77% ± 2.3) was in the HFD group which could be indicative of increased insulin resistance. Sea buckthorn fruit associated with the high fat diet improved the values of pAkt, by increasing it to 21.2% ± 3.1, $p < 0.05$. The value is close to the one from the control group, 23.2% ± 2.9.

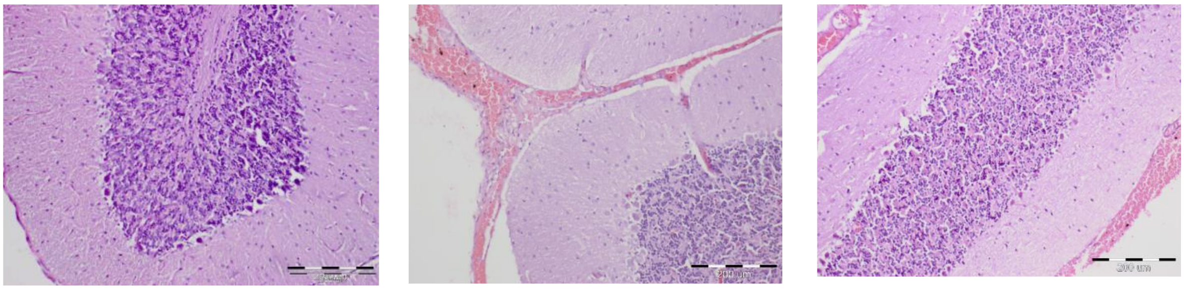
The haematoxylin eosin staining of the brain and of the cerebellum samples from the HFD rats showed disturbed histopathological features while in the HFDSB group, the Sea buckthorn fruit had neuroprotective effects. So, in the HFD group, sections through the central nervous tissue showed focally neurons with intensely basophilic cytoplasm, karyopyknosis, delimited by active glial cells and marked subdural congestion while in the HFDSB group, only passive hyperaemia of the subarachnoid vessels was observed.



(a) Control group *(b) HFD* *(c) HFDSB*

Figure 2.
Immunohistochemical examination with pAKT antibody in rat gastrocnemius muscle, 40x

In Figure 3, in the HFD group, the histopathological examination (haematoxylin-eosin dye) of the cerebellum showed karyopyknosis. The neurons from the the Purkinje cell layer of the cerebellar cortex had basophilic cytoplasm with condensation. In the HFDSB group, in the cerebellar cortex, the karyopyknosis was rarely seen.



(a) Control group, 100x *(b) HFD, 100x* *(c) HFDSB, 100x*

Figure 3.
The histopathological aspect of the cerebellum haematoxylin-eosin dye, x20

Culture cell studies demonstrated that flavonoids emulated neurotrophic activities by promoting neuronal cell differentiation through activating the PI3K/Akt protein [12]. In obese high fat diet mice, isorhamnetin, a flavonoid present in the Sea buckthorn fruit had a protective effect on cognitive impairment by increasing neurotrophic factors in the brains, lowering microglial over activation and down-regulating inflammatory cytokines in the serum and in the brain. The mechanism of action for isorhamnetin in the mouse brain is based on its capacity to inhibit the expression of the JNK, p38 and the NF-κB proteins [29].
Additionally, the Sea buckthorn polysaccharide (I) could enhance learning and memory functions and alleviate pathological impairments in Alzheimer's disease mice. The proposed mechanisms involve modulation of oxidative stress and inflammation. So, in the brains of Alzheimer disease mice, this polysaccharide increases the antioxidant enzymes as Superoxide dismutase and Glutathione peroxidase and reduces the tumour necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) levels. The Nrf2 (Nuclear factor erythroid 2-Related Factor 2) and the TLR4 (Toll-like receptor 4) transcription factors are involved in the signalling pathways generated by this polysaccharide [30].

In our study, the Sea buckthorn associated to the high fat diet decreased the increased values of glycaemia. The Sea buckthorn compounds are capable of increasing the clearance of the glucose from the bloodstream into muscle and adipose tissue, allowing the pancreatic β cell protection [31]. Due to the palmitoleic oil rich concentration, the Sea buckthorn has a potential to diminish skeletal muscle insulin resistance [32, 33]. Furthermore, dietary Sea buckthorn has a positive effect on increasing muscle mass and improving muscle fibre size distribution [34]. It has been also shown that Sea buckthorn berry increases the potassium intake, influencing the muscle contraction and nerve signals in the body [35].
Sea buckthorn extracts or Sea buckthorn puree protect the cardiovascular system by reducing blood pressure, improving dyslipidaemia and dysglycaemia [36]. Also, it has therapeutic effects, preventing diabetes mellitus [10].
In our study, dyslipidaemia was present in the HFD group. The Sea buckthorn fruit alleviated dyslipidaemia, by lowering the serum triglycerides level and increasing the value for HDLc (Table II). Also, it improved the liver function markers like ALT, AST (Table II). Observing the results in the HFD group and drawing a parallel between the low percentage values of pAkt in

the skeletal muscle, the imbalance of serum adipokines (low adiponectin and increased resistin, Table II) and the amount of ectopic fat (Table I), it can be said that all converge towards insulin resistance.

Conclusions

Our study demonstrated that the Sea buckthorn fruit intake significantly reduced the negative effects of the high fat diet on skeletal muscle and brain tissue. No corresponding manifestations to adverse reactions, as bleeding, for Sea Buckthorn fruit were recorded. There are some limitations of the study because of the low number of rats, the absence of a mRNA assays and the absence of a behaviour test for the rats. In high fat diet Wistar rats, weight gain was accompanied by increased intervisceral fat and higher serum levels for glucose, triglycerides and resistin. Also, an important disturbance of the histopathological appearance (haematoxylin-eosin staining) of the skeletal muscle and brain tissue was shown. Immunohistochemical staining demonstrated insulin resistance which was suggested by decreased phosphorylation of the muscle Akt protein. Co-administration of the thawed Sea buckthorn fruit to the high fat diet had beneficial effects by improving the metabolism, by increasing the insulin sensitivity via the Akt protein pathway and by maintaining almost to normal of the histological features of the brain and skeletal muscle.

Conflict of interest

The authors declare no conflict of interest.

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