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Myrtus communis extract ameliorates high-fat diet induced brain damage and cognitive function

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ABSTRACT: Obesity causes cognitive weakening and increases the risk of neurodegenerative diseases. *Myrtus communis* extract (MC) has anti-inflammatory and antioxidant properties. In this study, it is aimed to investigate the effects of *Myrtus communis* on oxidative brain damage caused by a high-fat diet (HFD), using behavioral and biochemical parameters. Twenty- four Wistar albino rats (200–250 g) were divided into three groups. The control group (C) received a standard diet, while HFD groups were received HFD for 16 weeks. MC (100 mg/kg, oral) was given to the HFD + MC group for the last 4 weeks. At the end of the study, the novel object recognition test (NORT) was performed and the hippocampus and blood samples were collected. Acetylcholinesterase (AChE) and Na^+/K^+- ATPase activities, malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and reduced glutathione (GSH) levels were measured in the hippocampal samples and cholesterol levels were analyzed in sera. Findings have shown that NORT performance of the HFD group was reduced, while administration of MC prevents this reduction and in parallel, increased AChE and decreased Na^+ /K^+ -ATPase activities were ameliorated by administration of MC. Increased MDA and 8-OHdG levels observed in the HFD group, were decreased in the MC treated HFD group. Our results point out that MC has ameliorative effects on hippocampal oxidative stress and cognitive impairment in high fat nutrition-induced obesity.

KEYWORDS: Obesity; oxidative stress; *Myrtus communis*; acetylcholinesterase.

1. INTRODUCTION

Recently, obesity has become a serious health threat as one of the most important causes of morbidity and mortality worldwide [1, 2], and the increase in the consumption of high-fat foods is considered as one of the main reasons for the increase in obesity [3]. Obesity is associated with type-2 diabetes, insulin resistance, cardiovascular diseases, and certain types of cancer [4]. Studies have shown that increased body weight or central obesity are related to increased dementia incidence [5, 6].

Obesity is characterized by a low level of chronic inflammatory status [7]. Obesity-related inflammation, oxidative stress and mitochondrial dysfunction establish the groundwork for the development of neurodegenerative diseases [8-11]. Therefore, excessive adiposity is seen as a risk factor for diseases that cause cognitive impairment, such as Alzheimer's disease (AD) [10, 12]. Experimental studies suggest that conditions such as obesity, high-fat diet (HFD) consumption, and insulin resistance alter brain structure, neurotransmitters, and functions [10, 13]. Obesity and HFD cause learning and memory impairment, hippocampal neuronal cell degeneration and neuronal inflammation [14-16]. Since the HFD increases oxidative stress in the brain, diets rich in antioxidants, such as polyphenols could be protective against oxidative damage [17].

Myrtus communis largely distributed in the Mediterranean area is an aromatic and always a green plant [18]. *Myrtus communis* leaves contain essential oil (α-Pinene, 1,8-cineol, linalool, limonene), phenolic acids, tannins and flavonoids [19-21]. *Myrtus communis* L. leaves have been shown to have many promising biological effects, including anti-inflammatory [22], analgesic [22], antioxidant [22, 23], neuroprotective [23], anti-acetylcholinesterase [24] and anti-diabetic [25].

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In the light of the current literature, the purpose of this study is to examine whether *Myrtus communis* extract (MC) has beneficial effects on oxidative stress in the brain caused by HFD, using behavioural and biochemical parameters.

2. RESULTS

2.1. Animals body weight

At the beginning of the experiment (T1), there was no significant difference among the body weight of groups. At the end of the 12th and 16th weeks (T2 and T3, respectively), all groups body weights significantly increased when compared to the T1 time and, the HFD and HFD + MC groups body weights were found to be significantly higher than that of the C group (Table 1, p<0.001). However, HFD + MC group body weight was lower than HFD group at the 16th weeks (p<0.01).

Table 1. T1: At the beginning of the experiment, T2: At end of the 12th week, T3: At the end of the 16th week. C: Control, HFD: High fat diet, HFD + MC: High fat diet+*Myrtus communis* extract.

Body Weight	C	HFD	HFD + MC
T1	258 ± 6.2	278 ± 8.3	276 ± 6.6
T2	369 ± 6.5 ^{aaa}	490 ± 13.6 ^{aaa,***}	467 ± 17.2 ^{aaa,***}
T3	403 ± 9.7^{aaa}	554 ± 15.6 $^{aaa, ***}$	483 ± 13.1 $^{aaa, ***, ++}$

^{aaa} p<0.001 compared to T1 time, *** p<0.001 compared to the control group,
$^{++}$ p<0.001 compared to the HFD group.

2.2. Novel object recognition test (NORT)

According to the NORT data at the end of the experiment, while the difference score significantly decreased in the HFD group compared to the C group (p<0.01), it increased significantly in the HFD + MC group compared to the HFD group (p<0.05) (Figure 1). These results show that while impaired short term object recognition memory by HFD administration, is improved by MC treatment.

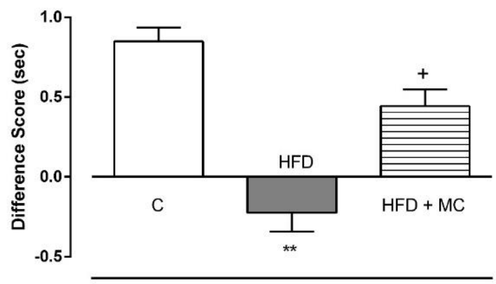

Figure 1. ''Difference score'' of the groups in the novel object recognition test. Each group consists of 8 animals. **p < 0.01: versus the C group, +p < 0.05: versus the HFD group. C: Control, HFD: High-fat diet, MC: *Myrtus communis* extract.

2.3. Biochemical analysis

While a significant increase was observed in the cholesterol level of the HFD group compared to the C group (p<0.05), no significant difference was observed between the HFD + MC group and C group (Table 2).

Table 2. Cholesterol level measurements in sera of the study groups.

	C	HFD	HFD + MC
Cholesterol (mmol/L)	47.7 ± 2.0	55.4 ± 1.6 *	48.5 ± 2.4

C: Control, HFD: High fat diet, MC: M*yrtus communis* extract * p<0.05 compared to the control group.

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AChE activity of the HFD group increased significantly compared to the C group (p<0.01). On the other hand, it was observed that AChE activity decreased significantly in the HFD + MC group compared to the HFD group (p<0.05) furthermore no significant difference was observed between the HFD + MC group and C group (Figure 2).

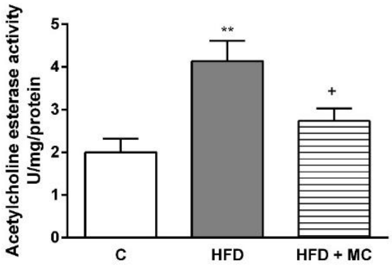

Figure 2. Hippocampal acetylcholinesterase (AChE) activities of the groups. Each group consists of 8 animals. * p < 0.05, **p < 0.01: versus the C group, +p < 0.05: versus the HFD group. C: Control, HFD: High-fat diet, MC: *Myrtus communis* extract.

The MDA level of the HFD group was significantly increased compared to the C group (p<0.001). It was observed that the MDA level of the HFD + MC group decreased significantly compared to the HFD group (p<0.01) (Figure 3a).

While a significant increase was observed in the 8-OHdG level of the HFD group compared to the C group (p<0.001), a significant decrease was observed in the HFD + MC group compared to the HFD group (p<0.05) (Figure 3b).

While the GSH level of the HFD group was significantly decreased when compared with the C group (p<0.05), no significant difference was observed between the HFD + MC group and the C group (Figure 3c).

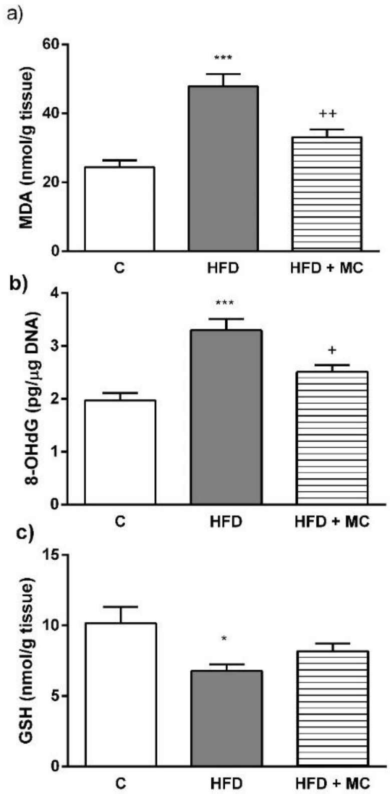

Figure 3. (a) Hippocampal malondialdehyde (MDA), (b) 8-hydroxyi-2′-deoxyguanosin (8-OHdG), (c) reduced glutathion (GSH) levels of the groups. Each group consists of 8 animals. * p < 0.05, ***p < 0.001: versus the C group, +p < 0.05, ++p < 0.01: versus the HDF group. C: Control, HFD: High-fat diet, MC: *Myrtus communis* extract.

Compared to the C group, the decrease in Na^+ /K^+ -ATPase activity (p<0.05) observed in the HFD group was significantly increased with MC treatment (p<0.05) (Figure 4).

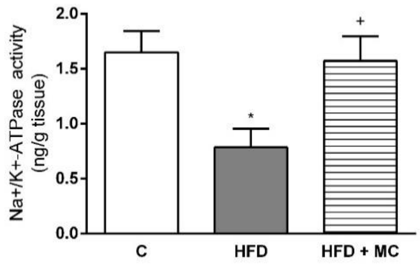

Figure 4. Na^+ /K^+ -ATPase activity levels of the groups. Each group consists of 8 animals. * p < 0.05: versus the C group, +p < 0.05: versus the HFD group. C: Control, HFD: High-fat diet, MC: *Myrtus communis* extract.

3. DISCUSSION

Our results showed that HFD caused to oxidative stress along with high MDA, 8-OHdG and low GSH levels and increased Na^+ /K^+ -ATPase activity in the hippocampus. In addition, increased AChE activity with HFD was found in the hippocampus. All these findings show that HFD causes oxidant damage in the brain, leading to the deterioration of memory function. On the other hand, a strong correlation was noted between MC treatment and decreased oxidative stress, AChE activity, and improved object recognition memory.

Similar to previous studies [26, 27] based on the body weight results we showed that the obesity model induced by the HFD successfully performed in the rats. Besides, a significant increase in cholesterol levels was observed in the animals fed with the HFD. Although MC administration had no significant effect on increased cholesterol level, these levels were similar to the control group in rats receiving MC treatment. This indicates that MC may have effects preventing the increase in cholesterol level. However, there is no information in the literature about the effectiveness of the *Myrtus* plant against obesity or high cholesterol level. Thus, further studies are needed to investigate the effect of MC on obesity and cholesterol levels.

Various studies have reported that HFD triggers oxidative stress in the brain and hippocampus [17, 28]. It has been shown that increased MDA levels and decreased GSH levels in the hippocampus [29], and brain [30] in rats receiving HFD. Similar to the aforementioned studies in this study HFD administration caused an increase in the MDA level and decrease in the GSH level. In the HFD + MC group, the MDA levels were effectively depressed with the MC showing the protection against oxidative damage. On the other hand, although the GSH levels were not significantly increased when compared to the HFD group, it was also not different from the control group. Kadıoğlu et al. (2020) have been shown to reduce brain damage in ovariectomized rats by MC treatment. In addition, it was stated in this study that cognitive functions were impaired in parallel with the damage, but improved with MC treatment [31]. This positive effect of MC treatment on memory was supported by Aykaç et al. (2019) and they have reported the decrease in cognitive functions with scopolamine was reversed with MC [24]. Similarly, our current study supports the above mentioned previous studies, and the memory loss caused by the oxidant damage caused by HFD in hippocampus were reversed by MC.

Reactive oxygen derivatives damage three major structures: lipids, proteins and DNA. 8-OHdG is measured as an indicator of damage to DNA [32]. Increased 8-OHdG levels in the hippocampus have been shown in rats previously exposed to HFD [33]. In this study, it was found that 8-OHdG levels were increased in rats with HFD, and MC treatment significantly reduced this oxidative damage marker. Our results support the probability that HFD may lead to DNA damage and weaken repair systems. *Myrtus* leaf extract has a good antioxidant capacity as it contains galloyl derivatives, flavonols and flavonol derivatives [18]. In experimental studies, antioxidant treatments have been shown to reduce 8-OHdG in the brain [34, 35]. Our results showing that MC treatment significantly reduces this oxidative marker is consonant with previous studies.

It is known that increase in AChE activity takes an important role in the pathogenesis of AD. Because of various side effects of currently available drugs, the search for new drugs is still continuing and especially inhibition of AChE activity is targeted. Different animals models are available for AD. Previous studies have provided evidence that obesity contributes to AD's pathophysiology. Mohamed et al. (2018) have been shown to increase in AChE activity in an obesity-induced Alzheimer's rat model caused by a HFD [13]. Similarly, in our study, AChE activity increased with HFD and this increase was suppressed with MC treatment. In an in vitro analysis Tumen et al. (2012) have reported that *Myrtus communis* extract inhibited AChE activity [23]. More recently, Kadıoğlu et al. (2020) have demonstrated that increased AChE activity in the brain was suppressed by MC in ovariectomized rats [31].

Na^+/K^+ -ATPase is located in the cell membrane and is responsible for the active transport of sodium and potassium ions. Oxidative stress targets Na^+/K^+ -ATPase system [36]. Na^+/K^+- ATPase depletion leads to depolarization in the membrane and ultimately excessive Ca^{2+} entry into neurons and causes neurotoxicity [37]. During oxidative injury, it has been shown that reactive oxygen species and free radicals damage to lipid bilayer and lead to depression in Na^+/K^+ -ATPase activity [38, 39]. In the present study, increased oxidative stress with HFD caused suppression in Na^+/K^+ -ATPase activity. On the other hand, MC treatment with antioxidant effect increased Na^+/K^+ -ATPase activity in the hippocampus by protecting the cell from oxidative damage at both the lipid layer and at the DNA level.

HFD rodent models reflect the obesity phenotype and cognitive deterioration [40]. Recognition of objects is considered to be a critical component of declarative memory [41]. Object recognition is deteriorated in human with neurodegenerative conditions or who have suffered from brain injury [41]. In the literature, it has been reported that HFD diet disrupts object recognition memory in various experimental studies [7, 42, 43]. It was demonstrated that mice fed HFD developed obesity after 13 weeks and the spatial memory and short-term object recognition memory were impaired, which was associated with hippocampal inflammation and oxidative stress [7]. In another study, it was suggested that increased inflammation in the cortex in mice receiving HFD diet caused disruption in the object recognition memory [43]. Our results indicated that object recognition memory was impaired with HFD as evidenced by significantly decreased difference score inNORT. Hippocampus is pivotal for object recognition memory [44]. Increased AChE activity and oxidative stress in the hippocampus may cause this deterioration of the recognition memory. On the other hand, MC therapy has been shown to improve deteriorated recognition memory in ovariectomized diabetic rats [31] and scopolamine-induced Alzheimer's model [24]. Also, Romani et al. (1999) reported that MC contained phenolic compounds such as catechin and myricetin-derived compounds as major constituents [20]. In previous studies, these compounds have been reported to have ameliorative effects in different traumatic brain injury models [45-49]. In addition, it was shown that catechin-derived compounds had the protective effects on the functions of brain in mice fed with HFD [50,51]. Therefore, the phenolic compounds found in leaves of *Myrtus communis*, especially catechin and myricetin-derived compounds, may be responsible for neuroprotective activity of the plant.

4. CONCLUSION

As a conclusion, MC is thought to have protective effects against hippocampal oxidative injury and cognitive impairment in a HFD induced obesity model.

5. MATERIALS AND METHODS

5.1. Animals and ethics

Thirty two Wistar albino rats (60 days, male, 200–250 g) were used. The rats were kept in cages (per cage 4 rats) under controlled temperature (22 ± 2°C) and humidity (60%–62%) levels for a 12 h light/12 h dark period. Feeding was done ad libitum. All study procedures were permitted by the Marmara University Animal Experiments Local Ethics Committee, Istanbul- Turkey (Protocol number: 98.2017.mar). Animals were obtained from the Experimental Animals Implementation and Research Centre, Breeding and Maintenence Unit (DEHAMER, Istanbul- Turkey).

5.2. Experimental protocol

Three study groups were determined: control (C, n=8) group, high-fat diet (HFD, n=12) group, and high-fat diet + *Myrtus communis* extract (HFD + MC, n=12) group. HFD and HFD + MC groups were fed with a HFD (oil content 40%, MBD Company) and tap water for 16 weeks [26]. From the 12th week the HFD + MC group was given daily MC (100 mg/kg) with orogastric gavage [24], every day at the same time and continued to be given for 4 weeks. The control group fed with standard rat diets and tap water for 16 weeks. The body weights of the animals were measured weekly at the beginning and 12th and 16th weeks of the experiment. AfterNORT, the animals were decapitated and hippocampus and blood samples were taken (Figure 5).

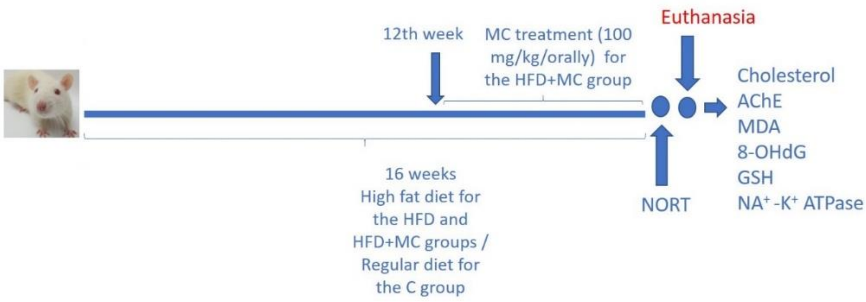

Figure 5. Summary of the experimental protocol.

5.3. MC preperation

Myrtus communis subsp. *communis* leaves were collected during flowering periods from the Antalya province of Turkey in April, 2018 and identified by Dr. Ahmet Dogan, a botanist of the Faculty of Pharmacy, University of Marmara. A voucher with number 22260 was deposited in the herbarium of School of Pharmacy, Marmara University, İstanbul, Turkey. The dried leaves were ground into fine powder by a grinder. A total of 100 g of powdered plant leaves material was extracted with ethanol (96%) using a Soxhlet device, and extraction was continued until the solution became colorless (up to 24 hr). The obtained ethanol extract was dried under a vacuum at 40°C. *Myrtus communis* extract (MC) powder obtained from the extraction of 100 g of powdered leaves was 30.02 g. MC was used for activity tests and kept in the dark at 4°C until use.

5.4. Cognitive tests

5.4.1 Novel object recognition test (NORT)

At the end of the experiment, NORT was applied to the animals for evaluation of the short-term recognition memory function [52]. NORT has two stages as familiarization and test stages. One day before the test day, each of the rats was placed on the test device for 10 min for adaptation as known habituation. At the test day, in the familiarization stage of the experiment, the same two objects as known sample object were placed in the test apparatus (50 × 50 × 30 cm.) and the behaviours of the animals were recorded for 5 min. This procedure was applied to all experimental animals and after 1 h, the test stage was started. In the second stage of the test, a new object was placed in the place of one of the objects and the behaviours of the animals were recorded again for 5 min. The results were given by comparison the time spent with the sample and the new objects. Object recognition test is based on the principle of spending more time interacting with the new than sample object, and this express as a positive difference score. The time difference spent with the new object was computation by the formula below and the results were expressed as a difference score [52, 53].

Difference score: Time spent with the new object−time spent with the sample object

5.5. Biochemical analysis

Cholesterol values in sera were assessed based on the manufacturer's manual and directions of the enzyme-linked immunosorbent assay (ELISA) kit designed for rats (YL Biotech Co. Shanghai, China).

Firstly 10% (w/v) hippocampal tissue homogenates with 0.1 M cold sodium phosphate buffer (pH 7.4) were prepared for ELISA tests.

AChE activity was assessed based on the manufacturer's manual and directions of the ELISA kit designed for rats (Elabscience, Wuhan, China).

MDA, 8-OHdG, and GSH levels were assessed based on the manufacturer's manual and directions of the ELISA kit designed for rats (Mybiosource, San Diego, USA).

Na^+ /K^+ -ATPase activity was assessed based on the manufacturer's manual and directions of the ELISA kit designed for rats (YL Biotech Co. Shanghai, China).

5.6. Statistical analysis

Statistical analysis were achieved using Graphpad Prism 6.0 (Graphpad Software, San Diego, CA, USA). All values are expressed as mean ± standard error (SEM). Values of groups were analyzed with one way analysis of variance (one-way ANOVA) followed by Tukey tests. Only NORT test outcomes were analyzed with Mann Whitney U nonparametric test. For all values, $p<0.05$ was considered to be statistically significant.

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

Ethics committee approval: All experimental protocols were performed according to the Marmara University Animal Experiments Local Ethics Committee, Istanbul- Turkey (Protocol number: 98.2017.mar) on December 4, 2017.

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