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Original Article

A possible role of nesfatin-1 and irisin in beneficial effect of *Capparis Ovata* extract on liver and kidney oxidative/ nitrosative status

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Abstract

Aim: In order to reveal the scientific evidence for its antioxidant and metabolic activities known in folk medicine, the present study was aimed to investigate the effect of bud and fruit parts of *Capparis ovata* plant on some antioxidant parameters and nesfatin-1 and irisin hormones in mus musculus mice for the first time.

Materials and Methods: The dry extract was obtained from bud and fruit parts of *C.ovata*. Twenty mus musculus mice were divided into two groups as control and *C.ovata* treatment group. *C.ovata* group was fed with 500 mg/kg *C.ovata* plant extract via gavage for 21 days. The nesfatin-1 and irisin levels in tissue were determined using enzyme-linked Immunosorbent assay methods. The total oxidant capacity (TOC), total antioxidant capacity (TAC), nitric oxide (NO), reduced glutathione (GSH) and gamma glutamyl transpeptidase activity levels were measured spectrophotometrically.

Results: Results showed that both irisin and nesfatin-1 levels were higher in liver and kidney of *C.ovata* group compared to the control groups. The liver TOC level was lower and the reduced GSH level was higher in group given *C.ovata*. The kidney NO levels were higher in *C.ovata* group. The extract increased synthesis of energy regulatory hormones and also exhibited antioxidant characteristics by reducing free radicals in the liver and affecting glutathione synthesis.

Conclusion: As a result, *C.ovata* can be used as a valuable phytotherapeutic agent in processes associated with the energy regulation and oxidative stress related many disease conditions.

Keywords: Antioxidant; caper; energy regulatory hormones; glutathione; nitric oxide

INTRODUCTION

Caper (*Capparis* spp.), a perennial shrub plant with a large natural distribution, is used as food and in traditional medicines in order to treat various diseases. Caper can be used for animal feed, preventing soil erosion and landscaping, and all parts (whole plant, roots, flowers, leaves, fruits, buds and seeds) of the Caper plant were used for various medicinal purposes in ancient times. Ancient Egypt and Arabs used this plant to treat liver, kidney, stomach, and skin diseases, while Romans used it to prevent parasites, while the Greeks used it to prevent headaches and toothache (1). *Capparis* species exhibit different pharmacological activities. In addition to the known antirheumatic, aphrodisiac, tonic, anti-microbial, anti-inflammatory properties of *Capparis* species, it is stated that the solid extracts of plant leaves, shoots and roots can be an effective cosmetic product in skin and hair diseases (2).

Caper has high levels of phenol, flavonoids, glucosinolates and alkaloids with high variety of phytochemical components. *C. ovata*, which tolerates drought and difficult conditions quite well, can grow in dry areas of Turkey Mediterranean, Central Asia, Greece, Cyprus, and India. The researchers found that the tolerance of *C. ovata* to drought depends on the antioxidant system that works even under high drought stress and its ability to reduce oxidative damage. *C. ovata* is also important economically because it can be used as drugs, cosmetics and food (3).

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Nesfatin-1, one of the new multifunctional neuropeptides, has been reported to play a role in regulation of food intake and anxiety responses (4). Irisin is a hormone that promotes the use of energy by transforming white fat tissue into brown fat tissue, defined by Bostrom et al. for the first time (5). No previous study investigating the effect of *Capparis* species on energy regulators nesfatin-1 and irisin hormones was reported. There are also no data available about hormones and antioxidant system in response to this specie. Therefore, the effect of methanol extract of *Capparis ovata* on antioxidant parameters such as total oxidant (TOC) and antioxidant capacity (TAC), nitric oxide (NO), reduced glutathione (GSH) and gamma glutamyltranspeptidase (GGT) activity levels and recently identificated, nesfatin-1 and irisin hormone levels, was investigated in mus musculus mice, for the first time.

MATERIALS and METHODS

Subjects

In the study, eight weeks old twenty male/female mus musculus mice were used. Prior to the study, a permission was obtained from the Local Ethics Committee of the Animal Experiments of Mehmet Akif Ersoy University (MAE-HADYEK/93773921-142/09.09.2015). The animals were maintained in special cages without any contact among the groups and all groups were fed with ad libitum-balanced diet. They were kept in a 12 hours light and 12 hours dark cycle at constant room temperature (19-21°C).

Experimental Design

The subjects were divided into two groups: (1) control group (n=10) and (2) experimental groups (n=10). The control group received oral physiological saline solution by gavage for 21 days and fed on a normal diet. The other group received only 500 mg / kg *C. ovata* plant extract by gavage for 21 days. Subsequently, at the end of the 21st day, the subjects were euthanized by cervical dislocation under anesthesia. The liver and kidney tissues were homogenized using cold lysis buffer containing 10 µg/mL aprotinin (from 10 mg/mL stock in water; stored at -20°C). Liver and kidney tissues were stored at -45°C until analyzed.

Preparation of *Capparis ovata* Plant Extract

The buds and fruits of *C. ovata* were obtained from a local herbal shop in Kayseri Province (Gul Food and Agricultural Products) Turkey. Methanolic extracts of the buds and fruits were prepared according to the method described by Ozkan et al (6). After collecting the bud and fruit parts of *Caparis* ovata plant, they were allowed to dry under suitable conditions. The dried *C. ovata* fractions are ground to a powder and then extracted with methanol in a Soxhelet apparatus for 8 hours. Then, the solvent in the extract will be evaporated at 40°C under vacuum with the help of rotary evaporator and dry extract will be obtained. The extract will be dissolved in saline and applied to the subjects by gavage.

Biochemical Analysis

Determination of Nesfatin-1 and Irisin Levels

The nesfatin-1 and irisin levels in tissue samples were determined using commercial mouse enzyme-linked Immunosorbent assay (ELISA) kits. The protein concentrations of the tissue samples were analyzed by the Bradford method using bovine serum albumin (BSA) as the standard, at 595 nm (7).

Determination of Total Antioxidant and Total Oxidative Capacity Levels

TAC levels of liver and kidney tissues were measured on Bio-Tek Eon auto analyzer using a Rel assay test kit (Rel Assay®, Gaziantep, Turkey). A stable antioxidant standard solution called the trolox equivalent was used for calibration. The trolox equivalent is a vitamin E analog, and the reaction rate could be adjusted with it. Antioxidants in the sample reduce dark blue-green colored 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample (8).

TOC levels of liver and kidney tissues were measured on Bio-Tek Eon auto analyzer using a Rel assay test kit (Rel Assay®, Gaziantep, Turkey). This method was calibrated with hydrogen peroxide. Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are present in reaction medium. The ferric ion makes a colored complex with chromogens in an acidic medium. The color intensity is related to the total oxidant molecules present in the sample at 530 nm (8).

Determination of Nitric Oxide Level

Nitric Oxide concentrations were determined with the chemical method in tissue samples. Tissue samples were de-proteinized with 10% zinc sulphate. Total NO (nitrate and nitrite) concentrations were measured colorimetrically by acidic Griess reaction (9).

Determination of the Reduced Glutathione Level

GSH analysis in the liver and kidney tissues was performed according to the Beutler method (10). Briefly, all proteins that do not carry the sulfhydryl (-SH) group in the samples were precipitated and the absorbance of the yellow complex resulting from the reaction of the proteins containing the -SH group with 5,5′-Dithiobis-(2-nitrobenzoic acid) was read at 412 nm in spectrophotometer.

Determination of Gamma Glutamyltranspeptidase Activity

Gamma glutamyltranspeptidase activity in tissue samples were determined commercial kits via colorimetrically (TML®, Ankara, Turkey).

Statistical Analysis

Statistical analyses were performed in triplicate and average values with standard deviation (mean±SD) are reported. The statistical significance was evaluated using the SPSS 16.0 software package (SPSS ver. 16.0 for windows professional edition). A one-way analysis

of variance (ANOVA) was performed and was followed by Duncan's test to estimate the significance at the 5% probability level.

RESULTS

The nesfatin-1 and irisin levels in the liver and kidney samples were investigated (Figure 1). The results obtained showed that both irisin and nesfatin-1 levels of the group given *Capparis ovata* were higher in liver (respectively, p<0.05 and p <0.001) and kidney tissues (both p <0.001) compared to the control groups.

Biochemical findings revealed that the liver TOC level was lower (p<0.05) and the reduced GSH level was higher (p<0.05) in the group given *Capparis ovata* compared to the control. There was no statistically significant difference in the liver TAC, NO and GGT activity levels between the groups.

The kidney NO levels were higher (p<0.001) in *Capparis ovata* group than in control. There was no difference between the groups in terms of kidney TAC, TOC, reduced GSH and GGT activity levels.

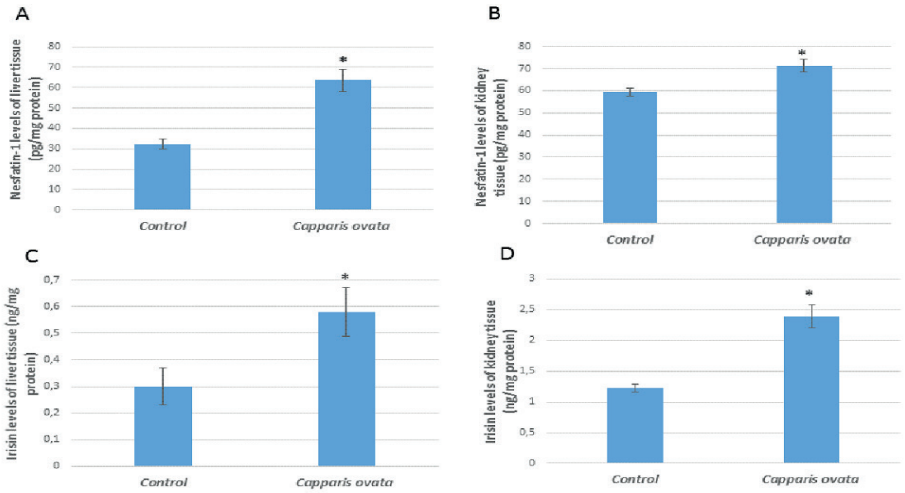

Figure 1. Tissue Nesfatin-1 and Irisin Levels. Nesfatin-1 levels of liver (A) and kidney (B). Irisin levels of liver (C) and kidney (D). *Compared to control group, p<0.001 (A, B, D), p<0.05 (C)

Table 1. Biochemical analysis results

Parameters	Tissue	Control (n=10)	*Capparis ovata* (n=10)	p
TAC (mmolTrolox Equiv./mg protein)		1.076±0.0336	1.0197±0.0414	Ns
TOC (µmol H_2O_2 Equv./mg protein)		31.305±2.176	25.219±1.469	<0.05
NO (nM/mg protein)	**Liver**	10.201±1.204	10.124±1.200	Ns
Reduced glutathione (nmol/mg protein)		24.138±0.415	26.705±1.044	<0.05
GGT activity (IU/mg protein)		1.251±0.227	0.552±0.208	Ns
TAC (mmolTrolox Equiv./mg protein)		1.150±0.032	1.165±0.059	Ns
TOC (µmol H_2O_2 Equv./mg protein)		18.272±0.822	18.245±0.731	Ns
NO (nM/mg protein)	**Kidney**	3.174±0.398	6.305±0.634	<0.001
Reduced glutathione (nmol/mg protein)		28.250±0.397	28.937±0.438	Ns
GGT activity (IU/mg protein)		14.67±0.980	11.981±1.183	Ns

Ns: Non-significant

DISCUSSION

All parts of the Caper aromatic plant are stored in salt, vinegar and brine and used as a complement to foods such as meat, salads, pasta, cheese and olives. The chemical and bioactive components of roots, seeds, leaves, buds and fruits of caper were examined by several researches (3,11-13). To provide scientific evidence for its antioxidant and metabolic activities known in folk medicine, the present study was aimed to investigate the effect of bud and fruit parts of *Capparis ovata* plant on some antioxidant parameters, nesfatin-1 and irisin hormones.

Caper seed oil has been reported to be an important source of fatty acids and has a content of linoleic and palmitic acid, particularly oleic acid (12,14-16). In a previous study, the arginine and aspartic acid content of these *Capparis* species was reported (15.1 and 7.7 g, 16 g N^{-1}, respectively)

(16). The fruits and buds of this species contain high amounts of potassium, phosphorus, magnesium and calcium and the seeds are rich in carotenoids, vitamin C and tocopherols with three isoforms (α-tocopherol, γ-tocopherol and δ-tocopherol) (3,17-19). Tlili et al. (1) reported that commercial caper contains significant quantities of caretinoid (pro-vitamin A), tocopherol, rutin (vitamin P) and total phenolic compounds and remarkable amounts of vitamin C, as bioactive compounds. *Capparis ovata* is a good source of energy with its high vitamin content, mineral and amino acid composition. *C. ovata* fruits was shown to be oleic (39.64%), palmitic (9.85%), stearic (2.38%), linoleic (21.38%), and linolenic acid (0.43%) (14,16).

Haifa Aichi-Yousfı et al. (20) compared the antioxidant activities of six taxa of the genus *Capparis* collected in Tunisia (*C. aegyptia, C. orientalis, C. ovata* subsp. ovata, *C. sicula* subsp. *sicula*, *C. spinosa* subsp. *spinosa* var. *spinosa* and *C. zoharyi*) and reported differences in the composition and content of the polyphenols and flavanoids and the antioxidant activities among these species. According to DPPH and ABTS test results, aqueous extracts of *Capparis ovata* subsp. ovata and *C. zoharyi* had the highest antioxidant activity in both tests. Bonina et al. (21) in them in vivo and in vitro tests have determined that lyophilized and methanolic extract of *Capparis spinosa* exhibits significant antioxidant / radical scavenger activity. In addition, major components of this species were found to be some flavonols (kaempferol and quercetin derivatives) and hydroxycinnamic acid derivatives (caffeic acid, ferulic acid, p-cumaric acid, and cinnamic acid). Germano et al. (22) have reported antioxidant activity due to phenolic content of the methanolic extract of *Capparis spinosa* L. buds. In a recent study, it was reported that *Capparis spinosa* seed extract decreased the level creatinine, urea, and uric acid (as markers of kidney injuries) following cisplatin administration and decreased AST and ALT (as markers of liver injuries) activities following CCL_4 administration in Swiss albino male mice. Antioxidant, protein and lipid oxidation results showed that the plant has a comprehensive antioxidant capacity (23).

Ozkur et al. (24) examined *Capparis* seeds under drought stress and concluded that *C. ovata* increased drought tolerance by reducing oxidative damage. They found that SOD, CAT and POX activities were higher in stressed seedlings and glutathione reductase was the highest on the 14th day of drought stress. In another study, p-methoxy benzoic acid obtained from methanolic soluble fraction of aqueous extracts of *Capparis spinosa* L. was determined to possess antihepatoxic activity against carbon tetrachloride and paracetamol induced hepatoxicity in vivo and thioacetamide and galactosamine induced hepotoxicity in vitro (25). This study showed that *Capparis ovata* significantly reduced TOC level in the liver. However, liver TAC and kidney TAC and TOC levels did not change. Reduced glutathione and glutathione associated metabolism have quite important roles in protecting cells from oxidative stress and other stress types. In the study *Capparis ovata* did not change in the kidney, while increasing the reduced GSH level of the liver. Although the hepatoprotective and nephroprotective effects of *Capparis ovata* are not yet known, its hepatoprotective effect may be due to the quality and quantity of phenolic compound, vitamins and other bioactive compounds.

Nesfatin-1, also referred to as toughness molecule and anorexigenic hormone, is associated with diabetes, obesity, anorexia nervosa, psychiatric disorders and neurogenic diseases. Nesfatin-1 in rats has been shown to be effective in regulating nutritional habits, food intake, body weight and glucose balance (26). The effect of hypothalamic toughness peptides on energy balance, obesity and glucose metabolism suggests that these peptides also have a regulatory role in normal and pathological processes (27). Nesfatin-1 has been reported to exhibit anti-inflammatory and gastroprotective effects by inhibiting the formation of pro-inflammatory agents and by balancing the oxidant and antioxidant system (28). However, in another study, it was determined that nesfatin-1 reduces sepsis-induced liver damage in rats without contributing to the antioxidant mechanism (29). In a study by Simin Nazarnezhad et al (30) nesfatin-1 has been shown to have protective effect by inhibiting oxidative stress against high glucose-induced cytotoxicity. In this study, *Capparis ovata* was increased nesfatin-1 levels of both liver and kidney tissues of mice. When liver biochemical results are examined, it can be predicted that increase of nesfatin-1 by *Capparis ovata* is related to antioxidant system. However, similar findings were not found in kidney tissue. Differently, *C. ovata* significantly increased nitric oxide levels in kidney tissue ($p<0.001$). Nitric oxide is a signaling molecule that has important roles in almost every biological system and has been shown to act as an endocrine molecule in recent years (31). NO produce in higher amount during inflammation by iNOS and activated by cytokines, known as primer defense system (32). During inflammation, NO increases and reacts with superoxide anions leading to formation of peroxinitrite radical (33) and this peroxinitrite radical leading to increase in lipid peroxidation and formation of free radicals (34). It was determined that *C. ovata* inhibited the NO radical in a dose-dependent manner and exhibited reducing power activity (35). In hydrophobic environments, such as biomembranes, NO reacts with radical species, acting as antioxidant and breaking lipid peroxidation processes (31). Szlachcic et al (36) indicated that nesfatin-1 increased the release of excessive NO and promoted mRNA expression of proinflamatory iNOS by activating cNOS expression. In their study, it was concluded that Nesfatin-1 has a strong protective effect on the stomach of rats exposed to water immersion and restraint stress and these effects are shown by NOS-NO system. It is thought that *Capparis ovata* increases NO production and NO acts as antioxidant. Few studies have demonstrated the relationship between nesfatin-1 and the antioxidant system. However, we can foresee that Nesfatin-1 and irisin levels may be related to NO production based on the results of the study, but this situation needs to be explained with further studies.

Irisin, a recently identified hormone, plays pivotal roles in energy expenditure by converting white fat tissue into brown fat and in oxidative metabolism (5). Treatment with exogenous irisin improved liver function, reduced liver necrosis and cell apoptosis, and relieved inflammatory response after hepatic I/R. Bi et al (37) show that irisin treatment (250 µg/kg, intravenous) was reduced liver MDA levels and was increased GSH-Px and SOD activities in male C57BL/6J mice. They found that treatment reduced hepatic damage by promoting mitochondrial biogenesis and alleviating oxidative stress. It has been reported that irisin reduces ROS production in hepotocyte cells and increases ROS formation in skeletal cells. Serum irisin has been shown to be negatively correlated with hepatic glutathione levels (38,39). Recent studies demonstrated that irisin could reduce oxidative and nitrative stresses and protect endothelial cells in type 2 diabetes (40). There was no previous study showing a direct relationship between irisin and nitric oxide. However, in this study, there was a positive correlation between irisin level and nitric oxide. When the control and *C.ovata* groups were examined, the irisin changes in tissues were similar to those of nesfatin-1. In addition to increasing the level of irisin, *C. ovata* reduced total oxidant capacity and increased the level of reduced glutathione in the liver. In this study, it can be stated that *Capparis ovata* has hepatoprotective effect. However, further studies are needed to determine whether the effect of this extract increasing the level of iris is related to the antioxidant system.

CONCLUSION

The results of this first study showed that methanol extract of *Capparis ovata* plant increased synthesis of energy regulatory hormones, irisin and nesfatin-1. Also, *C. ovata* also exhibited antioxidant characteristics by reducing free radicals in the liver and affecting glutathione synthesis. Although we cannot explain this situation with the available data, *C. ovata* increased kidney nitric oxide level. Effect of *C. ovata* on these recently discovered hormones and antioxidant system indicates that it can be used as a valuable phytotherapeutic agent in processes associated with the energy regulation and metabolic disorders in addition to cosmetic and nutritional purposes in the future.

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Competing Interests: The authors declare that they have no competing interest.

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Ethical Approval: The study protocol and ethics were approved by the Local Ethics Committee of the Animal Experiments of Mehmet Akif Ersoy University (MAE-HADYEK/93773921-142).

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